HEREDITY, VARIATION, AND THE APPEARANCE OF DIVERSITIES DURING THE VEGETATIVE REPRODUCTION OF *ARCELLA DENTATA*[1]

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INTRODUCTION

The problem

In the vegetative reproduction of rhizopod Protozoa conditions for the study of heredity, variation, and the method of evolution are more

[1] This work was done at the Zoölogical Laboratory of the JOHNS HOPKINS UNIVERSITY. The writer is indebted to the members of the Zoölogical Department for many courtesies, and is especially grateful to Professor H. S. JENNINGS for his valuable counsel.

simple than in any other animals. The body of Arcella is a single cell, which is as simple as any animal organism it is possible to obtain for such studies. During vegetative reproduction the two offspring at each division consist each of one-half of the parent. There are thus no heritable variations due to amphimixis, and if changes occur that are of evolutionary significance they may be easily recognized. During the past four years several reports have appeared, notably those of MIDDLETON (1915), JENNINGS (1916) and ROOT (1918), that cast some doubt upon the conclusions of earlier workers, that heritable diversities do not occur within a "pure line," that is, that the genotype is constant. Work of this sort is crucial, since it tests one of the requirements of evolution, i.e., that heritable diversities should appear that are not due to the mixture of factors from several individuals. The present contribution adds one more species of Protozoa in which there is an apparent inconstancy of the genotype during vegetative reproduction.

Advantages of Arcella for genetic studies

Arcella dentata was chosen as the material for this investigation because it possesses an unusually large number of favorable characteristics (fig. 1.)

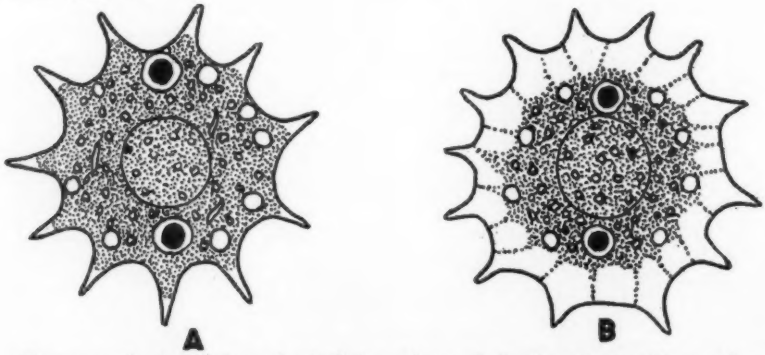

FIGURE 1.—**A**, aboral view of a typical specimen of *Arcella dentata* with 11 spines and a diameter of 116 microns. Imbedded in the cytoplasm (dotted) are 2 nuclei, a number of contractile vacuoles, and smaller food material. × 310.

B, aboral view of a specimen just after division. This specimen had 13 spines and measured 138 microns in diameter. × 310.

(1) It multiplies vegetatively and rapidly so that many generations may be obtained in a short period of time.

(2) It possesses definite morphological characters that are easily measured and not modified by growth.

(3) Environmental factors have no influence upon these characters after division is completed.

(4) Its characters are heritable but variable.

(5) The shell may be preserved easily, thus furnishing a permanent record.

(6) It is large enough to be conspicuous when placed under a Greenough binocular.

(7) The young are much lighter in color than their parents and hence there is no danger of confusing parent and offspring.

(8) The examination of living specimens is simple, because they attach themselves by their pseudopodia to the substratum and move very slowly.

(9) The almost transparent shell reveals the nuclei, chromidia, contractile vacuoles and other bodies within the living animal—especially in young specimens.

(10) Individuals will withstand severe operations and halves and even quarters will survive and reproduce provided they contain a nucleus.

Characters studied

The characters reported upon in this paper are (1) the number of spines, and (2) the diameter of the shell, measured between the bases of the spines. Other characteristics available for study are the size and shape of the spines (figure 5), the rate of fission, and the color of the shell.

Cultural methods

Arcella dentata, according to LEIDY (1879) and others, is rare when compared with *A. vulgaris* and *A. discoides;* nevertheless, an abundant supply was obtained from the lily pads in a pond on the campus of the JOHNS HOPKINS UNIVERSITY at Homewood, Baltimore.

The cultural methods used were similar to those employed by JENNINGS (1916) in his work on Difflugia. Water plants from nearby ponds were brought into the laboratory and the diatoms, algae, and ooze covering them were washed off by shaking in a bottle. The coarser materials were strained out and then the water containing the finer particles was transferred with a pipette to concavities in hollow-ground slides. As the work progressed, it was found that the Arcellas were not so apt to become covered by sediment if the culture slides were prepared from three to twelve hours before needed. During this time the particles sink

to the bottom, forming a fine film upon which the animals creep about and feed. It was also discovered that the Arcellas were more active and multiplied more rapidly if the pond water was diluted with an equal amount of distilled water. Thus in each cavity were placed two drops of the pond water containing food particles and two drops of distilled water. The brownish Arcellas are quite distinct against the grayish film of sediment on the bottom of such a culture, and there is no danger of contamination due to the inclusion of "wild" specimens that might occur in the food material.

New cultures were prepared, on the average, every three days, and the Arcellas were transferred to them with a capillary pipette. The cultures were examined almost every day and the young were removed to separate slides soon after they were produced. The number of the individual was then written with pencil on the rough surface of the slides. The cultures were not covered with cover glasses but were kept from drying by placing them in moist chambers consisting of large stender dishes. Each of these dishes accommodated eleven slides, or twenty-two specimens.

Specimens were easily found in the cultures with the help of a Greenough binocular and were then more closely examined under a compound microscope with the use of a water immersion objective.

For keeping pedigree records the system used by JENNINGS was adopted. This system was described by JENNINGS (1916, p. 415) as follows:

"For keeping pedigrees, records are kept in card catalogues, a card being devoted to each individual. The system may be illustrated as follows: The original 'wild' individual is given a number—say 21. Its first progeny is called 21.1, its second 21.2, etc. The first progeny of 21.1 is 21.1.1, its next 21.1.2, etc. Thus in later generations we have cumbrous labels such as 326.1.4.2.2.3.2.1.2.2.2.1.1.2. These labels must be written on the slide and its corresponding card. On the card is written a brief description of the individual, including the number of spines, and any peculiarity that will distinguish it from its progeny. Whenever an individual reproduces, that fact, with its date, and the number of spines of the progeny, is entered on the card of the parent, and a new card is written for the progeny. The long labels become troublesome, but each gives the full pedigree and relationship of the individual, and with the card records of all, the entire history may be reconstructed"

It was found desirable in certain cases to substitute for some of the longer labels one or more letters, such as **A, B, ED, SS,** etc.

Fission in Arcella

The process of fission in Arcella has not been described in detail and some of the stages were not observed during the course of this work. When ready to divide enough cytoplasm is protruded from the mouth of the shell to secrete a new shell; this cytoplasm lies against the substratum beneath the parent shell and thus the process of shell formation can not be observed from above. It seems probable that the spines of the shell are secreted around pseudopodia which extend out from this cytoplasmic mass. A number of specimens were examined after the new shell had been secreted but before division had taken place. In every case most of the cytoplasm was in the parent shell and only a small plug of cytoplasm extended into the new shell as shown in side view in figure 2. Cytoplasm then gradually flowed into the new shell until each shell contained approximately an equal amount. The cytoplasmic strand extending between the mouth openings of the two shells then grew thinner and finally broke and the parent and offspring moved apart sideways. There was no immediate swelling of the cytoplasm within the shells, which were therefore only about half full, as shown in figure 1B.

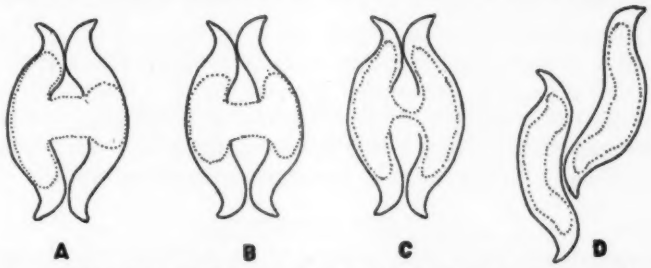

FIGURE 2.—Side views of division stages of *Arcella dentata*. The parent is at the left; the offspring at the right. The extent of the protoplasm is indicated by the dotted lines. The intervals between the stages are as follows: between **A** and **B**, 7 minutes; **B** and **C**, 3 minutes; **C** and **D**, 10 minutes. × 207.

Parent and offspring

The method of division of Arcella with respect to the shell, presents several difficulties not encountered in the study of shellless Protozoa. When it divides, the protoplasm apparently divides into two equal parts, i.e., the living substance of the parent passes over into that of the two daughters, but whereas one of the daughters is provided with a new shell, the other continues to live in the parental shell. Throughout this

paper I have referred to the latter as the parent and the former as the offspring. If a specimen has 12 spines, this number must be due to two factors, (1) the hereditary constitution of the protoplasm of its parent and (2) the environmental conditions during or preceding the formation of its shell. It is thus safe to conclude that the number of spines it possesses is a good index of its hereditary constitution, since it contains one-half of the parental protoplasm that secreted the shell and the environment was kept relatively constant. However, we have found that the young often vary from the parent in spine number and the problem thus arises as to whether the protoplasm of a 12-spined parent that has just given rise to a 10-spined offspring really corresponds to the shell in which it is confined or is a "square peg in a round hole." On this account a number of correlation tables were made up from data of the immediate or first progeny of each parent. When these tables were compared with those in which later offspring were included, it was found that the results were so nearly identical that no further attention was paid to this apparent difficulty. It seems probable that heritable diversities do actually appear among the progeny of a single individual, but the diversities are so small and the number of offspring belonging to other than the first division that were recorded were so few that very little difference results from their inclusion in correlation tables.

Counting generations

A second difficulty that arises because of the persistence of one-half of the parental protoplasm in the parental shell at the time of division is the counting of generations. This is of some importance since we measure the "permanency" of a characteristic in part by the number of generations through which it persists. Should the second offspring from a "parent" be considered as a member of the f_1 or of the f_2 generation, and should the parent after each fission be considered as belonging to the same generation as its last offspring? The accompanying parts of a pedigree (figure 3, **A**, **B**) show that such a parent may be considered a member of either generation, but really belongs to the f_2 generation. For convenience, however, all numbers of generations mentioned in this paper, unless otherwise indicated, have been counted according to the first method. It is a very simple matter to determine the number of the generations according to either method from the system of labelling employed. For example the specimen designated by the numerals 58.1.1.1. 4.2.1.1.1.1.3.1.3.1.2.4 belongs to the fifteenth generation according to the first method, this answer being found by counting the number of nu-

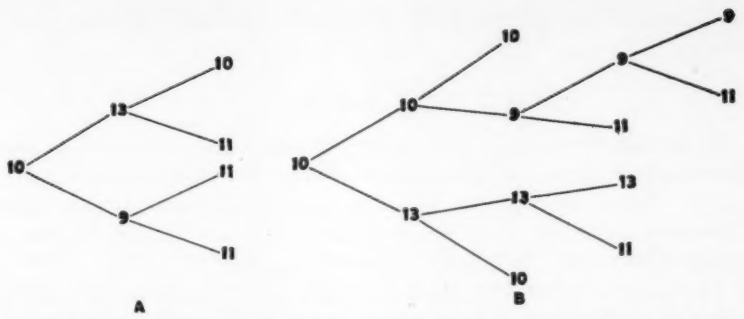

FIGURE 3.—Diagrams illustrating two methods of counting generations in Arcella. The diagram at the left, A, shows that the original parent with 10 spines gave rise successively to 2 offspring with 13 and 9 spines, respectively, and that each of these in turn gave rise to 2 offspring, the one with 13 spines to a first with 10 spines and a second with 11 spines, and the one with 9 spines to 2 successive offspring with 11 spines each.

In the diagram at the right, B, the parent after division is in each case shown as belonging to the same generation as the offspring.

merals after the family number, 58; or belongs to the twenty-seventh generation according to the second method, this answer being found by adding the same numerals together.

Preservation of specimens

It was found impossible in the time available, to preserve all the specimens, but a large proportion of them were saved for further study if this was found to be necessary. This was accomplished by transferring each individual to a separate drop of glycerine on a glass slide. One slide will hold without crowding about eight isolated specimens. The labels were written with India ink on the slides which were then placed in upright boxes where they keep for months without drying.

Length of life and number of progeny

No attempt was made to determine how many offspring may be produced by one parent nor how long a single parent will live. However, in looking over the records, it was found that specimen 58.1.1.1.1.1.2 1.1.1.1.1.2.2.1.1.1.2.1 was kept from December 20, 1917, to January 25, 1918, during which period it produced 18 offspring, and specimen 58.1.1. 1.4.2.1.1.1.1.3.1.1.1.1 was kept from December 7, 1917, to January 24, 1918, and gave rise to 21 progeny. There seems to be no reason why

these specimens might not have continued to live and reproduce indefinitely.

Computations

In the tables presented in this paper the coefficients of correlation were determined by means of the formulae used by JENNINGS (1916, pp. 416-417). The actual pedigrees, however, are perhaps better evidence than correlation tables of what has really occurred, and as many parts of pedigrees have been included as seemed advisable.

The specimens that form the basis of this report were obtained during a period of 174 days. The average number of generations studied was 69 and the average length of a generation was 2.5 days. In all, measurements of 6474 specimens are recorded. Of these 5557 belonged to the single family No. 58; 746 were secured from 70 small families; and 171 were collected directly from the pond.

VARIATIONS IN SPINE NUMBER AND DIAMETER OF SHELL IN A "WILD" POPULATION

The principal problem that this investigation was designed to solve is, Do variations in heritable characteristics that may occur in the progeny of a single specimen reproducing vegetatively lead to temporary or permanent diversities that would account for the diversities present in a "wild" population?

We must, of course, determine first what the variations are in such a "wild" population. Accordingly a large number of specimens of *Arcella dentata* were collected from various parts of the pond where they were

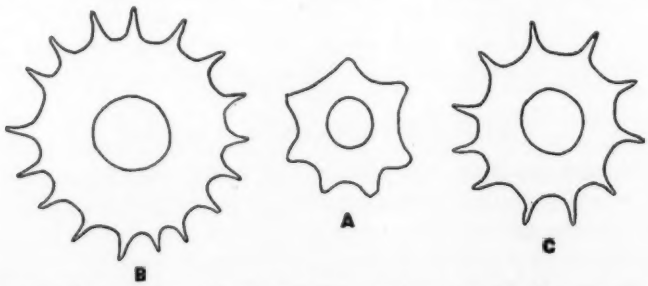

FIGURE 4.—Three specimens from a "wild" population of *Arcella dentata:* **A** was the smallest specimen found, possessing 7 spines and a diameter of 73 microns, **B** was the largest, with 17 spines and a diameter of 150 microns, **C** was in the modal class and near the mean, with 11 spines and a diameter of 116 microns. × 207.

FIGURE 5.—Some variations in the sizes and shapes of the spines of *Arcella dentata*. × 420.

present in great abundance. These were found to vary in size, and in number and length of spines. Some of these variations are indicated in figures 4 and 5. The extremes with respect to spine number were 7 and 17; and those with respect to diameter of the shell were 73 microns and 150 microns. No study was made of the variations in the shape or size of the spines, but such variations were incidentally noted and some of them are shown in figure 5.

TABLE 1

Correlation table for spine number and diameter of the shell in a "wild" population of Arcella dentata collected from a pond at Homewood, Baltimore, on Sept. 20, 1917. The unit of measurement is 4.3 microns. Correlation, .325 ± .060.

Diameter	Spine number 7	8	9	10	11	12	13	
23	1							1
24			1	2	1			4
25			2	12	4	1	1	20
26			3	12	10	3		28
27			4	12	7	2		25
28			3	4	5	5		17
29				2	1			3
30								0
31								0
32								0
33						1	1	2
	1	0	13	44	28	12	2	100

Table 1 shows the variations in spine number and diameter of the shells of 100 wild specimens taken at random from a large series collected on September 20, 1917, from the pond at Homewood and figures 6 and 7 give the same data in the form of curves. As regards spines, the mode is 10 and the mean 10.42, and for diameter the mode is 26 units and the mean 26.51 units. Specimens were found with spine numbers

of 8, 14, 15, 16 and 17, but these did not happen to appear in this sample of 100. A coefficient of correlation of .325 ± .060 shows that there is a significant relation between spine number and diameter in these or-

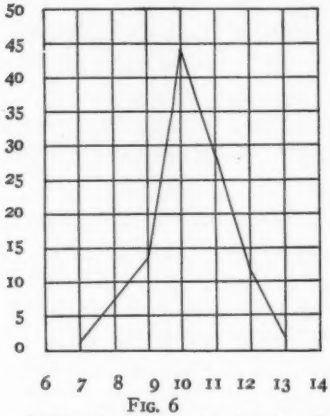

FIG. 6

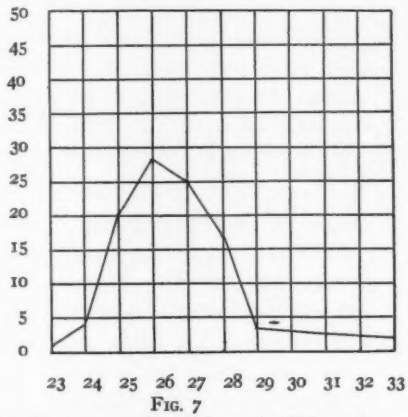

FIG. 7

FIGURE 6.—Curve showing the distribution of spine numbers of 100 "wild" specimens of *Arcella dentata* collected on Sept. 20, 1917, from a pond at Homewood, Baltimore. The ordinates are numbers of specimens and the abscissae numbers of spines.

FIGURE 7.—Curve showing the distribution of diameters of the 100 specimens recorded in figure 6. The ordinates are numbers of specimens and the abscissae diameters. The unit of measurement is 4.3 microns.

ganisms. Frequently irregular specimens were encountered (figure 12) principally among those with a low spine number; pedigree work indicates that many of these irregularities are due to environmental factors. They undoubtedly account for the high mean diameter of 26.46 of the group of 13 specimens with 9 spines shown in table 2. With the ex-

TABLE 2

Mean diameters of 100 "wild" specimens of Arcella dentata of given spine numbers. The unit of measurement for diameter is 4.3 microns.

Number of specimens	Spine number	Mean diameter
1	7	23.00
13	9	26.46
44	10	26.22
28	11	26.50
12	12	27.50
2	13	29.00

ception of this group, the mean diameter of the specimens measured increases gradually with the increase in spine number, exhibiting a marked correlation between these two characteristics.

Conclusion

The variations, therefore, in a random sample of 100 "wild" specimens are from 7 to 13 in spine number and from 23 to 33 units in diameter of shell, each unit being equal to 4.3 microns, and there is a marked correlation between the number of spines and the diameter of the shell.

HEREDITY AND VARIATION IN SPINE NUMBER IN FAMILIES DERIVED FROM SINGLE SPECIMENS BY VEGETATIVE REPRODUCTION

The next questions to be answered are, What kinds of variations occur during vegetative reproduction and to what degree are they inherited? Are heritable characteristics similar or different among families ("pure lines") descended from different "wild" specimens? To what degree are these heritable characteristics correlated in each family? Are there in nature a number of diverse families with regard to a given characteristic and are the several measurable characteristics correlated or independent in the various families? How many diverse families are there?

One hundred and forty-nine living specimens were selected from collections of wild animals, mostly taken on September 21 and 22, and November 23, 1917, and isolated on culture slides. These specimens were selected on the basis of diameter and spine number so that a beginning might be made with animals differing widely in these characters, as well as with those nearer the modal condition. Of the 149 specimens, 59 gave rise to small families and 12 to larger families. All of these, except one, either died out or were killed off because they could not be taken care of; the one family, number 58, was kept, being selected for further work. Tables 3 to 20 include only data regarding spine number.

Table 3 shows the distribution of the variations in number of spines within twelve of the larger families obtained. These are arranged according to the mean spine number beginning with the lowest mean. The range is from 10.40 to 14.07. A large interval occurs between the mean 11.21 of family 41 and that of 13.71 of family 62. Probably a larger number of families would supply means that would fill up this gap. Possibly, however, families 62, 109, and 83 should be considered as belong-

TABLE 3

Distribution of the variations in number of spines within twelve families.

Number of family	Number of spines										Total number of specimens	Mean spine number	Coefficient of correlation
	7	8	9	10	11	12	13	14	15	16			
18	1	1	4	8	11	5					30	10.40	.004 ± .123
85		2	5	12	14	6					39	10.43	—.158 ± .105
80		1	3	11	9	2	2				28	10.50	.013 ± .013
30			22	11	9	5	11				58	10.51	.279 ± .082
79		4	2	11	10	11					38	10.57	.032 ± .001
81		1	1	8	17	5	1				33	10.81	.085 ± .117
17		2	17	31	39	45	12	3			149	11.04	.062 ± .055
59			3	2	7	6	2				20	11.10	.039 ± .151
41		4		1	22	8	4	2			41	11.21	—.176 ± .102
62			1	2	2	2	4	11	9	4	35	13.71	.725 ± .054
109					1	2	4	11	10		28	13.96	.183 ± .128
83						5	7	13	10	5	40	14.07	.312 ± .093
											539		

ing to a distinct race. In only three families, 30, 62, and 83, is there any evidence of correlation between the spine numbers of parents and offspring.

Figure 8 gives the curves for four families separately and for twelve

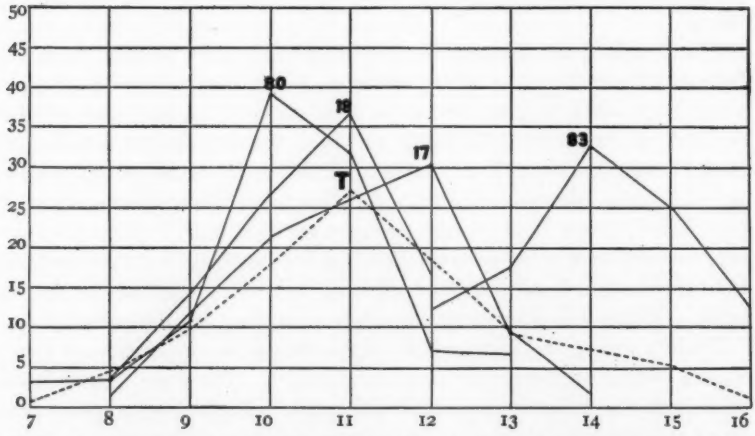

FIGURE 8.—Curves showing the distribution of the variations in spine number within four small families and for the total, T, of 539 specimens belonging to twelve families. The number with which each curve is marked is the designation of the family. The numbers of specimens in each family are shown in table 3. The ordinates are percentages; the abscissae, spine numbers.

families combined. Figures 9 and 10 are parts of the pedigrees of two of these families, showing quite clearly the great difference between them in spine number.

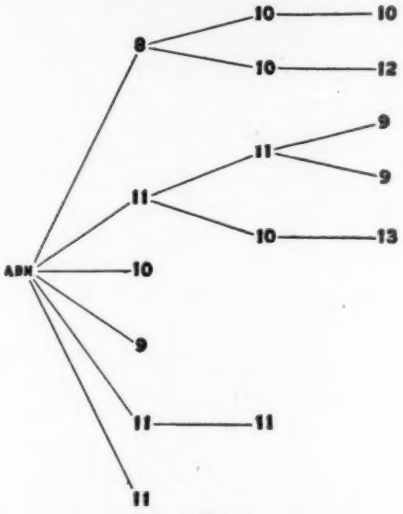

FIG. 9

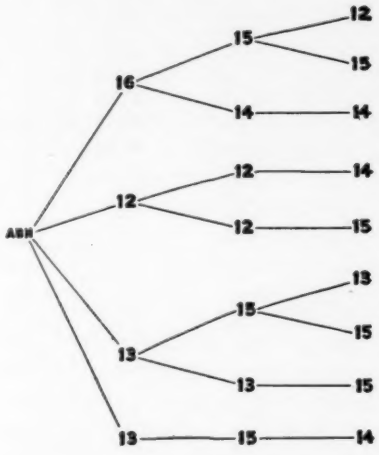

FIGURE 10

FIGURE 9.—Part of the pedigree of family 80 showing the number of spines of specimens belonging to the first 3 generations. The progenitor of this family (**ABN**; see fig. 12, **A**) was not normal and its abnormality was not inherited. It was selected for breeding purposes in order to determine whether or not abnormalities in *Arcella dentata* are inherited.

FIGURE 10.—Part of the pedigree of family 83 showing the number of spines of specimens belonging to the first three generations. This pedigree should be compared with that shown in figure 9. The abnormal progenitor of family 83 (see fig. 12, **B**) gave rise to normal offspring.

In all correlation tables the results of every fission were tabulated. Thus, when a specimen with 12 spines gave rise to one with 10 spines, the latter was recorded opposite number 10 in the parent column 12. Each offspring is counted but once as an offspring.

Table 4 presents the correlation for the inheritance of spines in the 70 small families taken together. This table consists of two principal sets of data; (1) the center of the table is occupied largely by offspring descended from specimens collected mostly on September 21 and 22, 1917. These have a mean spine number of a fraction less than 11.00. (2) The lower right-hand corner contains principally the descendants

TABLE 4

Correlation table for parents and offspring with respect to spine number within 70 small families. Correlation, .626 ± .015.

Progeny \ Parents	7	8	9	10	11	12	13	14	15	16	17	
7						1						1
8		2	1	1	3	4	1					12
9	1	1	12	11	13	9	1					48
10		9	12	28	40	36	10	1				136
11		9	16	37	44	44	9	4	1			164
12		2	5	17	38	25	25	7	4			123
13		2	3	3	9	17	24	11	9			78
14		1		1	4	24	20	29	18	6		103
15					2	11	14	11	10	12	1	61
16						2	1	4	8	4		19
17								1				1
	1	26	49	98	153	173	105	68	50	22	1	746

of very large specimens with many spines collected on November 23, 1917. This table shows that a high correlation (.626 ± .015) exists between the spine numbers of parents and progeny in this large population containing progeny derived from 70 "wild" specimens.

In table 5 is given the mean spine number for the 746 offspring produced by parents with different numbers of spines within the 70 families studied. It is clear that the spine number of the progeny approaches more closely that of the immediate parents than it does the mean of the race.

Among the small families that were studied were several whose curves

TABLE 5

Mean spine numbers of 746 progeny of parents of given spine numbers within 70 small families.

Number of specimens	Spine number of parents	Mean spine number of progeny
1	7	9.00
26	8	10.69
49	9	10.42
98	10	10.72
153	11	11.00
173	12	11.66
105	13	12.75
68	14	13.72
50	15	14.12
22	16	14.90
1	17	15.00

of distribution of spine numbers almost coincide. This, of course, might happen with families derived from specimens taken from widely separated localities, but in this case it seems probable that, since the specimens were all collected from a rather small pond and some of them from a single leaf, they were closely related and hence gave rise to families similar in the distribution of spine number.

Conclusion

Variations in spine number occur among the descendants of a single specimen during vegetative reproduction, and these variations are in part inherited. The hereditary constitution of different families obtained by vegetative reproduction from different "wild" specimens is different with respect to spine number. There is a marked correlation between parent and offspring with respect to spine number in three of the families (30, 62, and 83). The small differences between certain of the means, e.g., between those of the first five families listed in table 3, namely, 10.40, 10.43, 10.50, 10.51, and 10.57, indicate that, with respect to spine number, the heritable diversities between the different families may be extremely small and that a vast number of families exhibiting such heritable diversities may occur in a "wild" population. The large gap between the means 11.21 and 13.71 of families 41 and 62 would probably be bridged if a larger number of families were obtained, but this may represent a difference that might be considered of racial magnitude.

PERMANENT DIVERSITIES IN SPINE NUMBER AMONG THE DESCENDANTS OF A SINGLE SPECIMEN PRODUCED BY VEGETATIVE REPRODUCTION

At the end of 39 days from the time the rearing of the Arcellas was begun, their numbers had increased to such an extent that it was necessary to eliminate all but one family. The latter, family 58, was retained for the purpose of determining the degree of inheritance and the extent of diversities in a single large family, and to answer the following questions: Do variations that may appear in heritable characteristics in the descendants of a single specimen reproducing vegetatively lead to temporary or permanent diversities? May such diversities account for the heritable differences that appear between families obtained from "wild" specimens? In what way are such diversities produced?

The family derived from specimen 58 was saved for this work since its mean spine number was near the average of the species and its members seemed more viable than those of the other families. For convenience and also for the purpose of checking up environmental influence,

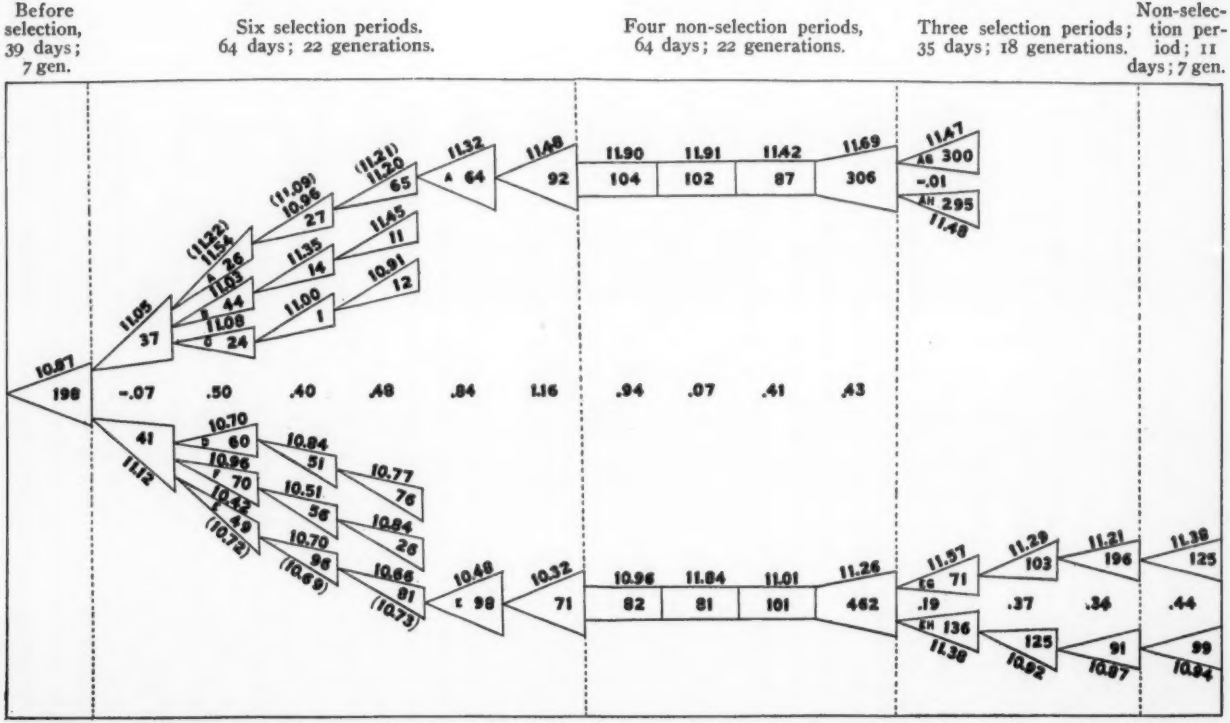

FIGURE 11.—Diagram showing the results of selection within the single family 58 for diversities in spine number. Each triangle or rectangle represents a period. The triangles are intended to indicate a gradual increase from one or a few selected individuals at the point at the left to a large number at the right. The rectangles indicate that the number of specimens was kept the same throughout the period. Within each triangle or rectangle is given the number of specimens studied during that period, and above each is given the mean spine number for the period. The upper series of triangles and rectangles give the data for the high line, and the lower series the data for the low line. Between the two series are the differences in mean spine number between the high and low lines. During the periods 2, 3 and 4, of the first series of selection periods, the high and low lines were made up of three branches each and labelled **A, B, C,** and **D, E, F,** respectively. The means for the three branches taken together during these periods are given in parentheses above the triangles in the high line and below the triangles in the low line.

the experiment was divided into a number of periods. The length of these periods was regulated so that enough progeny were obtained in each to give significant means. The periods were also determined in part by the fact that only a few hundred specimens can be taken care of by one investigator and when this limit has been reached a selection must be made. It may be noted at this place that several sudden large variations occurred in several of the periods, but the specimens exhibiting them, and their descendants are not included in the data presented in this part of the investigation but are given later under a separate heading. The data for the periods are given in tables 6, 14, and 18 to 21, and are indicated in the diagram on page 110 (figure 11), reference to which will make the following account clear.

Data before selection was begun

The progenitor of family 58 (figure 13, **A**) was collected on September 22. It had 12 spines. Because of faulty cultural methods it died after dividing only once, and a number of the early members of the family died from the same cause (figure 14). Later, specimens were very seldom lost, although a few died, some dried because of the evapo-

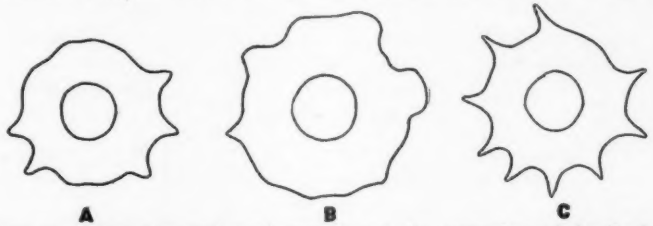

FIGURE 12.—Three abnormal specimens: **A** was the progenitor of family 80, and **B** of family 83; **C** apparently lacks one or two spines because of some mechanical obstruction in the environment. × 207.

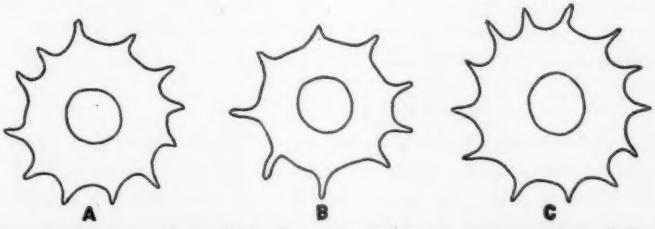

FIGURE 13.—Three specimens belonging to family 58. **A**, the progenitor of the entire family; **B**, a typical member of the low line; **C**, a typical member of the high line. × 207.

above the triangles in the high line and below the triangles in the low line.

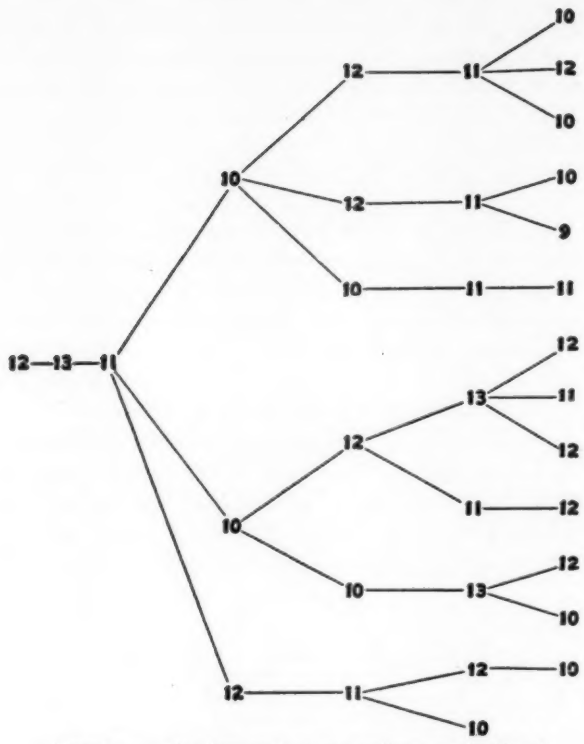

FIGURE 14.—The first part of the pedigree of family 58.

ration of the culture water, and several were lost during their transference from one culture to another. The first period was the longest and lasted for 39 days. During this time 198 offspring were produced with a mean spine number of 10.87. Table 7 gives the data for these 198 specimens. This table shows no evidence of correlation between parents and offspring, the coefficient of correlation being —.027 ± .048.

First selection period

On November 1 selection was begun. All specimens with 12 or more spines were selected as a basis for the high line and all those with 10 or less for the low line. These and all of their offspring were kept. The specimens selected for both the low and high lines were placed in the same moist chambers; were given the same proportions of food and water; and were all changed at the same time. Environmental condi-

tions were thus almost exactly alike for all the specimens at any particular time during the course of the work. At the end of 9 days this period was closed. The diagram on page 110 and table 6 show that the 37 progeny of the high line had a mean spine number of 11.05, and the

TABLE 6

Family 58. Results of selection for number of spines during selection periods 1 to 6. (Nov. 1, 1917, to Jan. 3, 1918.)

Period	Number of progeny	Correlation	High line		Low line		Difference
			Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (9 days)	78	.060 ± .076	37	11.05	41	11.12	—.07
2 (25 days)	273	.220 ± .039	94	11.22	179	10.72	.50
3 (9 days)	245	.186 ± .042	42	11.09	203	10.69	.40
4 (8 days)	271	.185 ± .040	88	11.21	183	10.73	.48
5 (7 days)	162	.403 ± .044	64	11.32	98	10.48	.84
6 (6 days)	163	.512 ± .039	92	11.48	71	10.32	1.16
64 days	1192					Mean difference	.55

TABLE 7

Family 58. Correlation table for parents and offspring with respect to spine number before selection was begun. Correlation, —.027 ± .048.

Progeny \ Parents	9	10	11	12	13	
9		3	4	1		8
10		9	19	15	2	45
11	5	12	21	25	6	69
12	1	16	19	14	6	56
13		8	5	7		20
	6	48	68	62	14	198

TABLE 8

Family 58. Correlation table for parents and offspring with respect to spine number in the first selection period (Nov. 1 to Nov. 9, 1917). Correlation .060 ± .076.

Progeny \ Parents	8	9	10	11	12	13	
8					1		1
9					1		1
10		4	8	1	7	2	22
11		4	10	1	10	2	27
12		3	5	1	9	2	20
13			3		3	1	7
		11	26	3	31	7	78

41 progeny of the low line had a mean spine number of 11.12. The diversity obtained was thus in the wrong direction, the mean spine number of the low line being actually greater by .07 than that of the high line. Table 8 indicates an entire absence of correlation during this period.

This result may have been due to the small number of offspring used, but later work makes it seem more probable that the selection was not successful, because high- and low-spined specimens were picked out regardless of the condition of their immediate and more distant ancestors,—a method that JENNINGS (1916) has shown to be inefficient under similar conditions in *Difflugia corona*.

Second selection period

The method of selection was changed at this time (November 10) and specimens with 12 or 13 spines that had parents, grandparents or other close relations with a high spine number were selected as progenitors of the high line, and similarly, specimens with 10 or less spines whose near relatives possessed low spine numbers, were selected as progenitors of the low line; that is, selection was based on past performance. All parents and offspring were kept during this period which extended over 25 days, from November 10 to December 4. Difficulty was encountered in the rearing of the Arcellas at this time. Many of them ceased to divide and all of the specimens became entangled in the food material to such an extent that they had to be extricated with needles. Large numbers died and the entire family seemed doomed, when a simple remedy was discovered. This was the dilution of the pond water, with which the cultures were made up, with an equal amount of distilled water. This procedure was suggested to me by the fact that DR. VERNON LYNCH of the JOHNS HOPKINS MEDICAL SCHOOL, had previously found that the addition of distilled water to his cultures was of benefit in the rearing of Amebas. Division immediately began again and no more trouble was experienced throughout the entire winter and spring seasons.

During the second selection period the 94 progeny in the high line possessed a mean spine number of 11.22, and the 179 progeny of the low line, a mean spine number of 10.72, or a divergence of .50. The mean spine number of the high line was thus .35 above the mean of the family before selection was begun and that of the low line was .15 below this mean. Table 9 shows the degree of correlation of parent and offspring during this period.

It was found that at the end of the second selection period all progeny in the high line were descended from three specimens, and similarly, all

those in the low line were descended from three specimens. The branches to which these specimens had given rise were from this time on designated as **A**, **B**, and **C** in the high line, and **D**, **E**, and **F**, in the low line, and the data for these six branches are indicated in the diagram on page 110. It is worthy of note that the mean spine number of each of the

TABLE 9

Family 58. Correlation table for parents and offspring with respect to spine number in the second selection period (Nov. 10 to Dec. 4, 1917).

Correlation .220 ± .039.

Progeny \ Parents	8	9	10	11	12	13	
8			1				1
9			5	4	5		14
10		1	34	23	9	3	70
11		7	40	43	20	2	112
12		1	11	28	22	5	67
13			1	3	4	1	9
		9	92	101	60	11	273

three subfamilies in the high line is greater than that of any of the three subfamilies in the low line, and that the means of all six subfamilies differ slightly from one another. Too much emphasis should not be attached to this but it suggests the possibility that these six subfamilies may represent six permanent heritable diversities in spine number.

Third selection period

At the end of the second selection period a few specimens were selected from each of these six branches on the basis of past performance and all parents and progeny were kept until the end of the period (9 days). As shown by the diagram on page 110, the three branches of the high line did not increase as rapidly as did those of the low line. During this period the mean spine number of the 42 offspring in the high line was 11.09, and of the 203 offspring in the low line, 10.69, giving a divergence of .40.

As during the second selection period, the mean spine number of each of the three branches of the high line is greater than that of any of the three branches of the low line. Table 10 shows the degree of correlation between parent and offspring during this period.

Fourth selection period

At the end of the third selection period a few specimens were selected from each of the three branches of the high line and from each of the

TABLE 10

Family 58. Correlation table for parents and offspring with respect to spine number in the third selection period (Dec. 5 to Dec. 13, 1917).

Correlation .186 ± .042.

Progeny \ Parents	8	9	10	11	12	13	
8			2				2
9		5	13	4			22
10	1	8	38	13	9		69
11	2	13	44	20	18	4	101
12	1	6	18	4	11	5	45
13			4	1	1		6
	4	32	119	42	39	9	245

three branches of the low line, on the basis of past performance.

All these specimens and their offspring were kept during the period of 8 days. As indicated in the diagram on page 110, the mean spine number of each branch of the high line was higher than that of any branch of the low line. The mean of the entire high line was 11.21 and that of the entire low line 10.73, giving a divergence of .48. Table 11 shows the degree of correlation between parent and offspring during the fourth selection period.

Fifth selection period

At the end of the fourth selection period, two branches, **B** and **C**, were eliminated from the high line, and two, **D** and **F**, from the low line. As

TABLE 11

Family 58. Correlation table for parents and offspring with respect to spine number in the fourth selection period (Dec. 14 to Dec. 21, 1917).

Correlation .185 ± .040.

Progeny \ Parents	8	9	10	11	12	13	
8							
9	1	3	9	3	3		19
10	1	13	28	16	11	3	72
11		19	43	23	23	5	113
12		5	16	13	18	4	56
13		1	2	6	2		11
	2	41	98	61	57	12	271

progenitors of the fifth period, a few specimens from branches **A** and **E** were selected on the basis of past performance.

Selection was also practiced *during* the course of the fifth period; all progeny in the high line with less than 12 spines were immediately elimi-

nated after being recorded and all specimens that persisted in giving rise to low-spined offspring were likewise eliminated; in a similar way all offspring in the low line with more than 10 spines were eliminated, and also all specimens that produced several high-spined progeny. All progeny were, of course, included in the data before they and their parents were eliminated. This method of selection was most effective. The mean spine number of the high line rose to 11.32, that of the low line decreased to 10.48, and the divergence increased to .84. Table 12 shows the degree of correlation between parents and offspring during this period, and figures 15 and 16 give parts of the pedigrees of the two lines at this time.

Sixth selection period

Selection at the beginning and during this period was carried on in

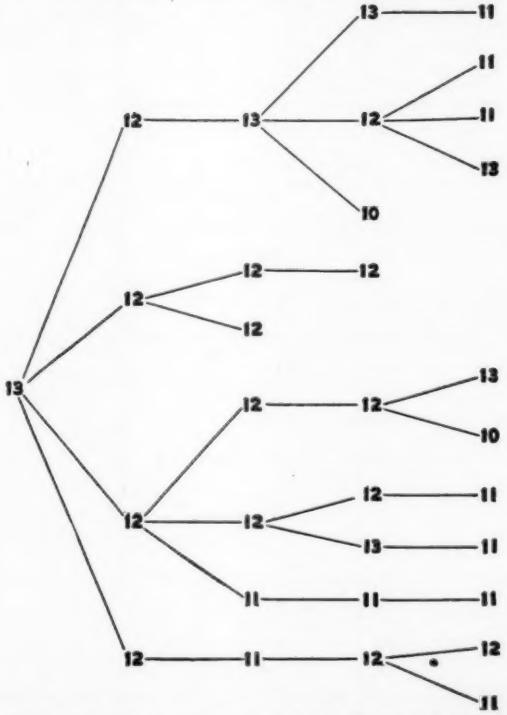

FIGURE 15.—Part of the pedigree of the high line of family 58 during the fifth selection period.

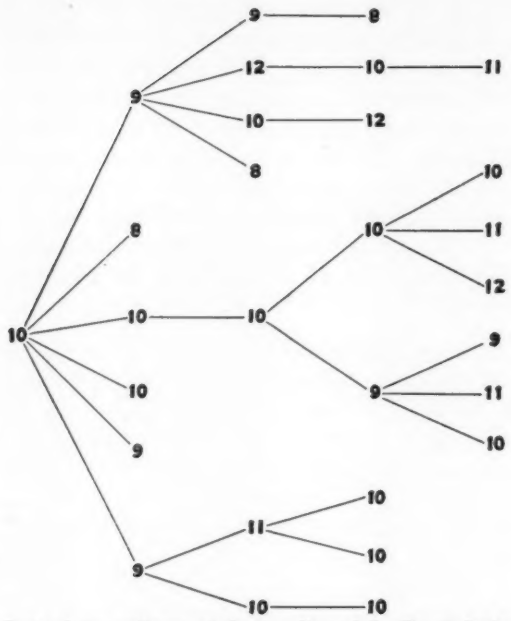

FIGURE 16.—Part of the pedigree of the low line of family 58 during the fifth selection period.

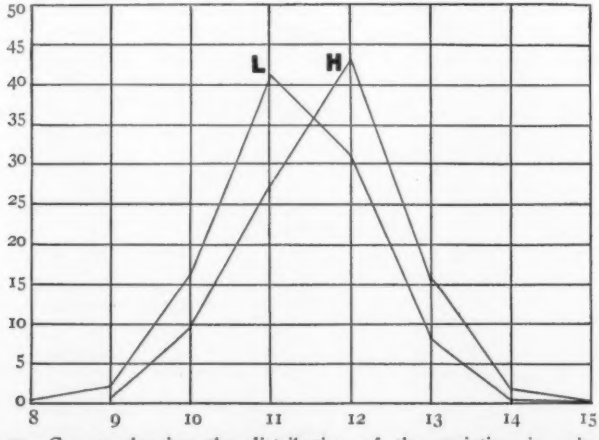

FIGURE 17.—Curves showing the distribution of the variation in spine number within the high and low lines of family 58 during the non-selection periods. The ordinates are percentages; and abscissae, spine numbers; H= the high line; L = the low line.

TABLE 12

Family 58. Correlation table for parents and offspring with respect to spine number in the fifth selection period (Dec. 22 to Dec. 28, 1917).

Correlation .403 ± .044.

Progeny \ Parents	8	9	10	11	12	13	14	
8		1	2	1				4
9	1	6	1		1			9
10	2	9	14	6	7	3		41
11	1	3	20	18	28	5		75
12	1	1	5	5	10	3	1	26
13				1	5			6
14					1			1
	5	20	42	31	52	11	1	162

the same way as during the fifth period. A rise in mean spine number to 11.48 in the high line, and a decrease to 10.32 in the low line gave a divergence of 1.16. Table 13 shows the degree of correlation between parents and offspring during this period.

Selection was discontinued at the end of the sixth period. The mean spine number had increased in the high line from 10.87, which was the

TABLE 13

Family 58. Correlation table for parents and offspring with respect to spine number in the sixth selection period (Dec. 29, 1917 to Jan. 3, 1918).

Correlation .512 ± .039.

Progeny \ Parents	8	9	10	11	12	13	14	
8		2	2					4
9	3	2	2	1		1		9
10	2	6	18	1	9	1		37
11		5	15	4	19	12	1	56
12		3	5	3	20	12		43
13				2	7	3	1	13
14						1		1
	5	18	42	11	55	30	2	163

mean of the family before selection was begun, to 11.48, a difference of .61, and had decreased in the low line from 10.87 to 10.32, a difference of .55. The average difference between the means of the high line and the low line during these six selection periods was .55.

Conclusion

It has thus been demonstrated that by means of selection, two lines can be obtained from a single specimen reproducing vegetatively, that differ in measurable, heritable characteristics. This difference may seem

slight but it is constant not only for the high and low lines, but also for the separate branches (**A**, **B**, **C** and **D**, **E**, **F**) into which these lines were divided, and hence is significant. The question now arises, Is this divergence temporary or permanent?

Non-selection periods

To test the permanency of the divergence obtained during the six selection periods of 64 days, a series of non-selection periods was inaugurated. Because it is impossible for one investigator to take care of more than a few hundred specimens at one time, the following method was used during the first three non-selection periods. Twenty-two representative specimens (the number that can be accommodated in one moist chamber) were taken from the high line, and 22 from the low line. Whenever these divided, the parent was eliminated and the offspring kept. Thus the number of specimens was kept down but no selection was practiced. Only immediate progeny obtained during the first three non-selection periods were thus recorded, but during the fourth period, several offspring from a single parent were included. The data obtained during the four non-selection periods are presented in figure 11, and in tables 14, 15 and 16. As the diagram on page 110 and table 14 show, there was an immediate decrease in the difference between the means of the high and low lines from 1.16 to .94 during the first non-selection period and to .07 during the second non-selection period. During the third period the difference increased to .41.

At the end of the third non-selection period, the high and low lines were allowed to expand; that is, *all* parents and offspring were kept. This was continued for 15 days and a very large number was obtained in

TABLE 14

Family 58. Spine numbers during the four non-selection periods (Jan 3 to Feb. 6, 1918).

Period	Number of progeny	High line		Low line		Difference
		Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (6 days)	186	104	11.90	82	10.96	.94
2 (7 days)	183	102	11.91	81	11.84	.07
3 (7 days)	188	87	11.42	101	11.01	.41
4 (15 days)	768	306	11.69	462	11.26	.43
35 days	1325				Mean difference	.46

TABLE 15

Family 58. High line. Correlation table for parents and offspring with respect to spine number in non-selection periods 1 to 4 (Jan. 3 to Feb. 6, 1918). Mean spine number of all (588) progeny, 11.94. Correlation, .170 ± .027.

Progeny \ Parents	8	9	10	11	12	13	14	15	
9			4		1				5
10		2	10	18	17	6	2		55
11		4	28	32	71	20	5		160
12		2	30	62	114	43	6		257
13		1	11	17	40	25	3	1	98
14			2	1	5	3	1		12
15					1				1
		9	85	130	249	97	17	1	588

TABLE 16

Family 58. Low line. Correlation table for parents and offspring with respect to spine number in non-selection periods 1 to 4 (Jan. 3 to Feb. 6, 1918). Mean spine number of all (737) progeny, 11.28. Correlation, .197 ± .024.

Progeny \ Parents	8	9	10	11	12	13	14	15	
8			1						1
9		1	6	4	4	2			17
10	2	8	30	47	27	7			121
11		8	48	135	92	19	1		303
12		2	50	82	72	21		2	229
13			6	20	19	17			62
14				1	1	1			3
15				1					1
	2	19	141	290	215	67	1	2	737

each line; 306 in the high line and 462 in the low. Again a difference between the two lines appeared—this time of .43. Since the divergence between the high and low lines persisted throughout the 4 periods of non-selection, representing 18 generations, the conclusion was reached that a permanent divergence had been obtained.

It will be noticed that the mean spine number increased in both the high and low lines during the first non-selection period. Thus that of the high line during the sixth selection period was 11.48 and in the first non-selection period was 11.90, and that of the low line during the sixth selection period was 10.32 and in the first non-selection period 10.96. The mean spine number of the high line increased only by .01 during the second non-selection period; whereas that of the low line increased by .88. A decrease in both lines occurred in the third non-selection period and an increase again in the fourth non-selection period. Several other

lines were being propagated at the same time and in these similar increases and decreases were noted during these periods. One of these lines consisted of specimens derived from a large Arcella collected on December 27, 1917, thus showing that these changes in spine number were not restricted to descendants of specimen 58. A similar increase in spine number was noted by JENNINGS (1916, p. 489) in *Difflugia corona*. The cause of these increases and decreases is not known but their appearance in all lines at the same time points to some environmental factor, probably the composition of the water or food. Some of the data for certain days during these periods (table 17) indicate very clearly the extent of these increases and decreases.

TABLE 17

Family 58. Table showing distribution of the spine numbers of progeny of high and low lines on three dates. On Dec. 31, the distribution was almost "normal" for the two lines. On Jan. 15 the spine number had increased in both, but more noticeably in the low line. On Jan. 18 a reduction in the number of spines in both lines to near the normal is indicated; and on Jan. 26, an increase again in both lines.

Date	Line	Spine number							Total number of progeny	Mean spine number
		8	9	10	11	12	13	14		
Dec. 31	High			1	13	17	5		36	11.72
	Low	3	2	18	8	5			36	10.27
Jan. 15	High			1	4	5	4		14	11.85
	Low				4	5	1		10	11.70
Jan. 18	High			2	3	7			12	11.41
	Low			4	4	3			11	10.91
Jan. 26	High				3	4	3	1	11	12.18
	Low			1	3	1	1		6	11.66

Divergences similar to those obtained in family 58 are no doubt occurring in nature at all times, and probably lead to greater differences there than those recorded above. Such differences as exhibited by families 80 and 83 (see figures 9 and 10) might have arisen in this way, and, while the means of certain branches from these lines might approach each other and even coincide, the lines as a whole would remain distinctly different, each possessing a different mean. (See figure 27.)

Selection within the high line

The problem that was next undertaken was to determine whether high and low lines could be obtained from each of the high and low lines al-

ready secured. Accordingly a few specimens were selected from the high line as progenitors of a new high line, and a few were selected from the high line as progenitors of a new low line. These selections were made, as formerly, on the basis of past performance, and all parents and progeny were kept during a period of six days. The results as in-

TABLE 18

Family 58. High line. Results of selection for high and low numbers of spines (Feb. 8 to Feb. 13, 1918).

Period	Number of progeny	High branch		Low branch		Difference
		Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (6 days)	595	300	11.47	295	11.48	—.01

dicated in the diagram on page 110 and in table 18 show that the mean spine number of the 300 specimens in the high line was 11.47 and that of the 295 specimens in the low line was 11.48—a difference of only .01. This slight result and lack of time necessitated the discontinuance of this experiment so that selection could be continued in the low line, but the data obtained from the latter leave no doubt that further work would have been successful.

Selection within the low line

Specimens with a high spine number and those with a low spine number were selected on the basis of past performance from those of the low line that were living at the end of the non-selection periods. These were reared during three selection periods of 4, 9 and 6 days respectively. Selection was practiced at the end of each period and also during the periods. As shown in the diagram on page 110 and in table 19 a divergence was obtained of .19, .37, and .34 respectively during the three selection periods. A non-selection period was then inaugurated lasting for 11 days (table 20). At the beginning of this period a representative group of specimens was selected in each line. A divergence of .44 resulted. Since the data thus obtained seemed to prove that two lines differing in their heritable characteristics had been obtained from the low line, the work with these lines was discontinued.

It seems probable also that divergences of a similar sort would be

TABLE 19

Family 58. Low line. Results of selection for high and low numbers of spines (Feb. 8 to Mar. 2, 1918).

Period	Number of progeny	High branch		Low branch		Difference
		Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (4 days)	207	71	11.57	136	11.38	.19
2 (11 days)	228	103	11.29	125	10.92	.37
3 (8 days)	287	196	11.21	91	10.87	.34
23 days	722				Mean difference	.30

TABLE 20

Family 58. Low line. Spine numbers during non-selection period (Mar. 2 to Mar. 13, 1918).

Period	Number of progeny	High branch		Low branch		Difference
		Number of progeny	Mean spine number	Number of progeny	Mean spine number	
1 (11 days)	224	125	11.38	99	10.94	.44

obtained if these two divergent branches of the low line were subjected to further selection as described above.

Thus a family containing all of the descendants of a single individual reproducing vegetatively would probably be found to consist of a large number of subfamilies, each differing slightly from the others in heritable characteristics. These divergent subfamilies would correspond in heritable characteristics to the small families derived from many "wild" individuals such as those described in part 3 of this paper (p. 105).

PERMANENT DIVERSITIES IN DIAMETER OF SHELL AMONG THE DESCENDANTS OF A SINGLE SPECIMEN PRODUCED BY VEGETATIVE REPRODUCTION

a. *Diversities in diameter of shell between the high and low lines of family 58*

The question with which we are concerned here is, Do permanent diversities in measurable heritable characteristics other than spine number

appear among the descendants of a single specimen reproducing vegetatively?

It was impossible to measure all of the specimens of family 58 that were examined for spine number, but at intervals during the progress of the work measurements were made of enough of the specimens to give significant means. The data obtained are presented in tables 21 to 24 and may be summarized as follows:

TABLE 21

Family 58. Table showing distribution of diameters of shells within the high and low lines during the non-selection period (Jan. 15 to Jan. 26, 1918.) The unit of measurement is 4.3 microns.

Line	Diameters							Total number of progeny	Mean diameter
	24	25	26	27	28	29	30		
High	1	5	25	37	48	10	1	127	27.26
Low	2	10	25	65	28	6		136	26.92

Difference, .34

Measurements were made of all progeny in both high and low lines from Jan. 15 to Jan. 26, 1918, a period covering parts of the second, third and fourth non-selection periods. Table 21 gives the distribution of the diameters; the total number of progeny and the means obtained. The mean diameter of shell in the high line was greater than that of the low line. The difference of .34 unit is not great, but is as large as could be expected considering the fact that the mean difference in spine number at the same time was only .41. As was to be expected from previous tabulations a high correlation was found to exist between diameter of

TABLE 22

Family 58. Correlation table for parents and offspring with respect to diameter of the shell in both high and low lines during the non-selection period (Jan. 15 to Jan. 26, 1918). The unit of measurement is 4.3 microns. Correlation, .489 ± .035.

Progeny \ Parents	24	25	26	27	28	29	30	
24		2	1					3
25		1	8	3	2			14
26	1	5	10	24	4			44
27		4	13	45	23	3		88
28		1	5	17	31	8	1	63
29				1	3	1		5
30								
	1	13	37	90	63	12	1	217

shell and spine number (table 22). We may conclude, therefore, that permanent diversities in the diameter of the shell, as well as in spine number, have appeared among the descendants of specimen 58 during vegetative reproduction. It is remarkable how constant these characteristics are among the progeny of a single specimen, but this very constancy emphasizes the importance of the differences observed.

b. *Diversities in diameter of shell between the high and low branches of the low line of family 58*

The next question is, Is there a difference in the diameter of the shell in the high and low branches of the low line of family 58 corresponding to the difference in spine number? Measurements were made of all progeny in these two branches from Feb. 14 to Feb. 23 and from Mar. 7 to Mar. 13, 1918. The distribution of diameters of shells, the total number of progeny, and the means for both these periods and for the two periods combined are tabulated in table 23. This table shows that

TABLE 23

Family 58. Low Line. Table showing the distribution of diameters of shells within the high and low branches of the low line for two periods (Feb. 14 to Feb. 23, and Mar. 7 to Mar. 13, 1918). The unit of measurement is 4.3 microns.

Branch	Date	Diameters						Total number of progeny	Mean diameter
		25	26	27	28	29	30		
High	Feb. 14-23	4	12	42	25	8		91	27.23
Low	Feb. 14-23	1	27	56	34	6		124	27.13
High	Mar. 7-13	2	18	38	29	5	1	93	27.21
Low	Mar. 7-13	8	28	20	15	5		76	26.68
High	Feb. 14-23 Mar. 7-13	6	30	80	54	13	1	184	27.22
Low	Feb. 14-23 Mar. 7-13	9	55	76	49	11		200	26.96

Difference .26

during the period from Feb. 14 to Feb. 23, the high branch had a mean shell diameter .10 units greater than that of the low branch; that during the period from Mar. 7 to Mar. 13 this difference was .53 units; and that when the two periods are combined the difference is .26 units. At the same time the mean difference in spine number was .40.

These data prove that there is a difference between the mean diameter of the shells in the high and low branches of the low line of family 58 corresponding to the difference between them in mean spine number. The positive correlation between these characters within this family is similar to that found in a "wild" population (compare tables 24 and 1).

TABLE 24

Family 58. Correlation table for spine number and diameter of the shell in both high and low lines during the non-selection period (Jan. 13 to Jan. 26, 1918). The unit of measurement is 4.3 microns. Correlation, .255 ± .042.

Spine number	Diameter 24	25	26	27	28	29	30	
9			2	2				4
10	1	2	13	20	14			50
11		7	16	36	24	5		88
12		4	5	27	22	4		62
13			1	3	7	3	1	15
14				2	1			3
	1	13	37	90	68	12	1	222

DIVERSITIES IN DIAMETER OF SHELL AND IN SPINE NUMBER DUE TO SUDDEN LARGE VARIATIONS ("MUTATIONS")

As stated above, the constancy in both diameter of shell and spine number during the vegetative reproduction of *Arcella dentata* is remarkable. The difference in diameter of shell between parent and offspring is seldom greater than 2 units (each unit being 4.3 microns) and that in spine number usually not more than 1 or 2. In four cases there appeared in the cultures under observation, differences in diameter of shell between parent and offspring that were so great as to be conspicuous. In all of these cases the parents belonged to the low line (**E**) and the offspring were smaller than their parents. Three of the branches established by these small offspring can be disposed of very briefly; the other two deserve more extended treatment.

Branch **EF**

Specimen **EF** was the ninth offspring of a parent (58.1.1.1.4.2.1.1.1 .1.1.1.1.1) that had nine spines and measured 25 units in diameter. **EF** also had nine spines but measured only 22 units in diameter. At the time **EF** appeared (December 22) the mean spine number of the low line (**E**) was 10.48 and the mean diameter 26.81 units. Part of the descendants of **EF** are shown in pedigree form in figure 18. The diameter of shell and spine number are small in the first generation, but increase to

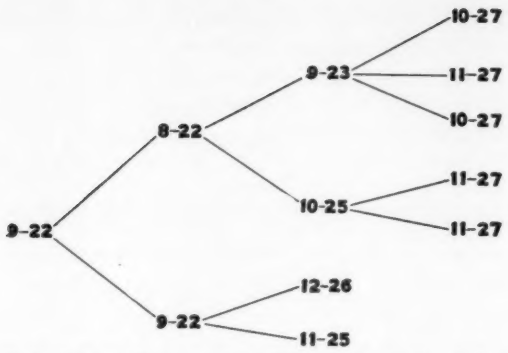

FIGURE 18.—Part of the pedigree of branch EF. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns.

the mean of the line in the third generation. The small diameter of EF was therefore only temporary and probably due to some environmental influence, and the small diameters of the first and second generations indicate that when the decrease in size is not due to a change in the germ plasm, it requires about three generations for the descendants of a very small specimen to reach the normal size.

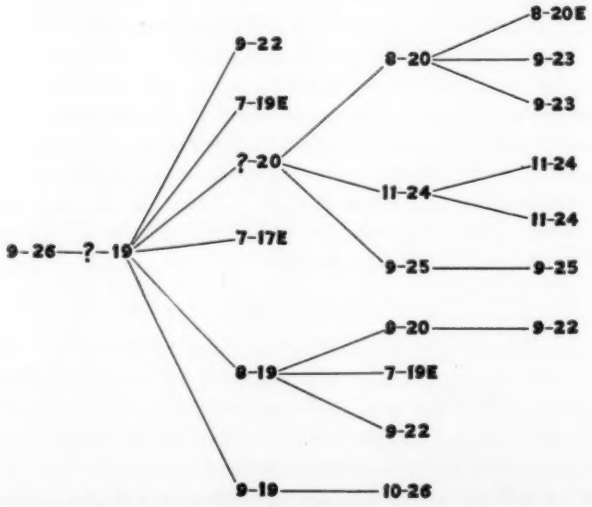

FIGURE 19.—Part of the pedigree of branch E421. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns.

Branch **E421**

The progenitor of this branch, whose full label is 58.1.1.1.4.2.1.1.1.1 .4.2.1., arose on December 8 from a specimen with nine spines and a diameter near the average of the parent line. Figure 19 gives part of the pedigree of this branch and shows that although small specimens still appeared in the fourth generation, there was a gradual increase in diameter and spine number that would probably have resulted in the attainment of a mean spine number of about 10.69 and a mean diameter of shell of about 26.81 which were those of the line **E** (on December 27, 1917), from which this branch was derived. It is, of course, possible that some of the sub-branches may have remained small, but lack of time necessitated the elimination of the entire branch.

Branch **EM**

Specimen **EM** was also a member of branch **E** of the low line. Its entire label is 58.1.1.1.4.2.1.1.1.1.1.1.1.1.3. It was regular in form, had 8 spines, and was only 18 units in diameter (figure 22, **B**); whereas the mean spine number of the low line at the time **EM** appeared (December 13) was 10.69 and the diameter (on December 27) was 26.81

TABLE 25

Family 58. Branch EM. Table showing the number of progeny and their mean diameter for ten generations (Dec. 13, 1917, to Jan. 19, 1918). The unit of measurement is 4.3 microns.

Number of generation	Number of progeny	Mean diameter
1	10	23.40
2	32	25.34
3	54	26.01
4	52	26.34
5	47	26.31
6	41	26.41
7	27	27.11
8	18	27.00
9	24	26.62
10	98	26.51
Total	403	

TABLE 26

Family 58. Branch EM. Correlation table for spine number and diameter of shell of 97 specimens. The unit of measurement is 4.3 microns. Correlation .368 ± .059.

Diameter	Spine number 8	9	10	11	12	
23		2	1			3
24		1	3	2		6
25	1	1	14	6	1	23
26	1	2	23	19	6	51
27			3	8	1	12
28				1	1	2
	2	6	44	36	9	97

units. The parent of **EM** had 10 spines and was 27 units in diameter. This parent gave rise to offspring normal in diameter and spine number both before and after **EM** appeared. Measurements were not made of the specimens but the spine number is indicated in the pedigree shown in figure 20.

By December 29, the descendants of **EM** had increased to 58 and it became necessary to eliminate some of them. Since the diameter and spine number seemed to increase at each succeeding generation, it was decided to try to get enough specimens in each of the first ten generations to furnish significant means for each generation. As tables 25, 26 and 27 show, **EM** produced ten offspring whose mean diameter was 23.40. There was a gradual increase in the mean diameter during the first four generations (figure 21) and then fluctuations until the tenth generation (January 19, 1918) when the work was stopped. During the period when these progeny were obtained (December 13, 1917, to

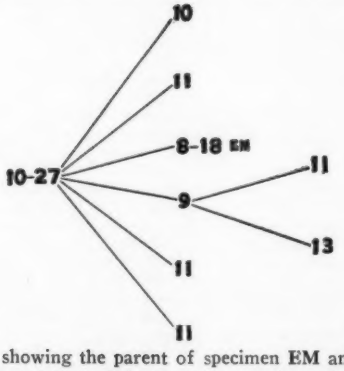

FIGURE 20.—Pedigree showing the parent of specimen **EM** and five of the sisters of **EM**. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns.

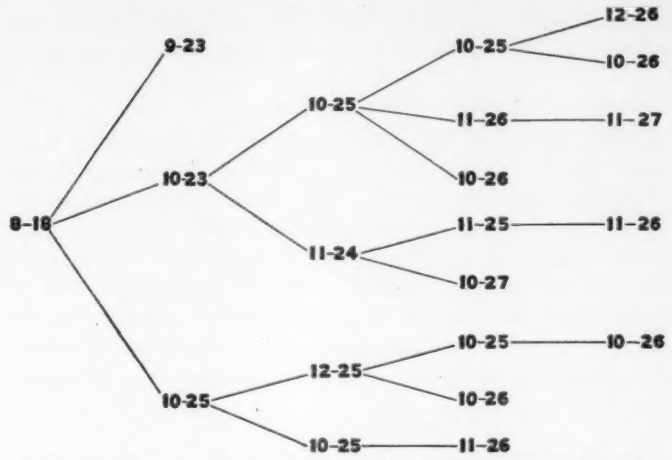

FIGURE 21.—The first part of the pedigree of branch EM. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns.

January 19, 1918) the mean diameter of the low line **E** from which **EM** arose was 26.81 units (on December 27, 1917) and the mean spine number 10.62. These data indicate that the divergence of **EM** and the first 4 or 5 generations derived from it was only temporary and that **EM** could not be called a mutation in the sense that its germ plasm had become permanently modified. As noted above in the case of branch **EF** (page 128) when for some reason a specimen appears that is markedly different in size from its parent, this decrease in size being due to some environmental factor, it seems to require several generations before the parental condition is regained. At certain times during the cultivation of branch **EM** sudden increases and decreases in the diameter and spine numbers of all the progeny occurred similar to those noted in the other

TABLE 27

Family 58. Branch EM. Table showing a notable increase and subsequent decrease in spine number and in diameter of the shell. The unit of measurement is 4.3 microns.

Date	Number of progeny	Mean spine number	Mean diameter
Feb. 2	9	11.22	27.55
Feb. 7	7	12.28	28.85
Feb. 9	8	10.87	27.50

branches of family 58 (see page 121). Table 27 gives the data for a few days during which a notable increase and subsequent decrease took place.

Branch ED

The work carried on with this branch was more extensive than with the other branches described above, and led to the greatest divergences that were discovered during the entire work. The specimen **ED** appeared on December 22, 1917. Its complete label is 58.1.1.1.4.2.1.1.1.1 .1.1.1.1.8. It was the eighth offspring of a specimen that was of average size and whose other offspring were of average size. **ED** itself is very close to the mean of the race, measuring 26 units in diameter and possessing 9 spines. The line from which it arose had a mean spine number at this time of 10.48 and a mean diameter of 26.81 units. **ED**

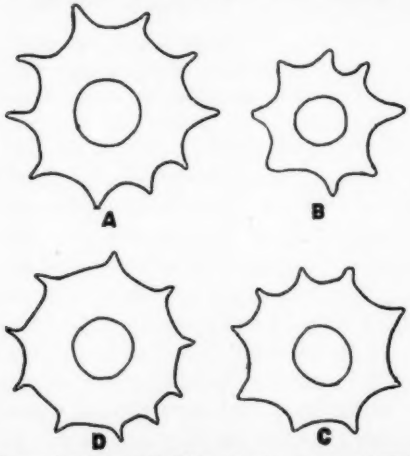

FIGURE 22.—Members of the branch **EM**. **A**, the parent of **EM** with 10 spines and a diameter of 116 microns. **B**, **EM** with 8 spines and a diameter of 77 microns. **C**, the first offspring of **EM**, with 9 spines and a diameter of 99 microns. **D**, a typical member of the fourth generation with 10 spines and a diameter of 116 microns. × 207.

produced 8 offspring with a mean diameter of 20 units and a mean spine number of 8.80. The diagram on page 133 (figure 24) gives a portion of the pedigree of this branch; figure 23 on the same page indicates graphically the principal phases of the work; and table 28 gives the mean diameters and spine numbers of the generations from 1 to 24.

All parents and offspring in this branch were kept for a period of 20

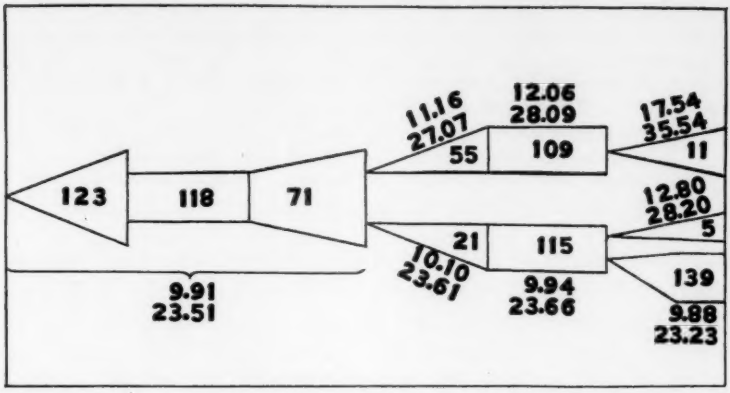

FIGURE 23.—Diagram showing data obtained during the cultivation of branch ED. The data are arranged as in figure 11.

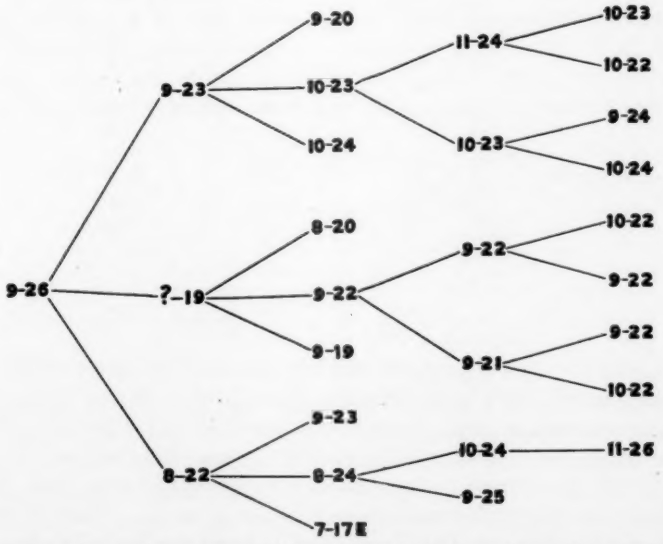

FIGURE 24.—Part of the pedigree of branch ED beginning with specimen ED. The number before each dash is the spine number; that after each dash, the diameter in units of 4.3 microns. The letter E indicates that the shell was empty.

days until there were 123 in all. Then a representative lot of 22 specimens were taken and for a period of 27 days all parents were eliminated

TABLE 28

Family 58. Branch ED. Table showing the mean diameter and mean spine number for generations 1 to 24 (Dec. 22, 1917 to Feb. 22, 1918). This table includes the large specimens belonging to the subbranch EDA, but not the members of the subbranches EDB and EDC. The unit of measurement is 4.3 microns.

Number of generations	Number of specimens	Mean diameter	Mean number of spines
1	8	20.00	8.80
2	12	21.75	9.25
3	28	22.78	9.74
4	28	23.57	10.10
5	20	24.30	10.00
6	22	24.13	10.54
7	15	23.60	9.66
8	10	23.50	10.10
9	9	23.88	10.22
10	13	24.00	10.30
11	9	23.44	10.22
12	8	23.12	9.37
13	6	23.83	9.33
14	6	23.33	9.66
15	14	23.57	9.57
16	24	23.50	10.08
17	19	23.42	9.84
18	6	24.33	9.33
19	15	24.53	10.33
20	15	24.00	10.06
21	11	23.73	10.00
22	8	23.37	10.25
23	5	24.00	10.40
24	1	24.00	9.00
1–24	312	23.51	9.91

as soon as they had reproduced and the offspring were kept. Then for 11 days both parents and offspring were kept. During these three periods, totaling 58 days, records were made of 312 offspring. These had a mean diameter of 23.51 units and a mean spine number of 9.91. During the same period the low line **E** from which **ED** arose had a mean diameter of 27.05 and a mean spine number of 10.99. There is thus a difference in diameter of 3.54 units and in spine number of 1.08.

In the fifth and sixth generations of this branch there appeared in one subbranch a remarkable increase in diameter and in spine number, as shown in the diagram on p. 137 (figure 26, **EDA**). This increase may be tabulated as follows:

Generation	Spine number	Diameter
1	8	22
2	8	24
3	10	24
4	11	26
5	13	31
6	16	32

Several empty shells were thrown by members of this subbranch, but empty shells were of rather frequent occurrence in all the lines studied and in the other lines did not cause any such remarkable changes. There were only three very large specimens produced; these measured respectively 31, 31, and 32 units in diameter, and possessed 13, 14 and 16 spines respectively. No further offspring were obtained from these nor from their progenitors although every care was taken to keep them in favorable cultural conditions.

On February 20 it was decided to try selection with those members of the branch **ED** that remained alive at that time. Four specimens were selected on the basis of past performance to start a high line and 4 to start a low line. The diameters and spine numbers of these were as follows:

High line		Low line	
Diameter	Spine number	Diameter	Spine number
24	10	22	9
25	11	22	10
25	10	22	9
26	12	19	9

During a period of 9 days fifty-five offspring were produced in the high line with a mean diameter of 27.07 units and a mean spine number of 11.16, and 21 offspring in the low line with a mean diameter of 23.61 and a mean spine number of 10.10 (table 29).

Twenty-two representative specimens were then taken from the high line and all of the 21 in the low line were kept. All parents were eliminated as soon as they reproduced and the offspring retained. The number of offspring and means obtained during this period of 13 days are given in table 29.

Four specimens were then selected from the high line for the purpose

TABLE 29

Family 58. Branch ED. Table showing mean diameter and mean spine number during one period (Feb. 20 to Feb. 28, 1918) and a subsequent non-selection period (Mar. 1 to Mar. 13, 1918). The unit of measurement is 4.3 microns.

Period	Number of progeny	High branch			Low branch			Mean difference	
		Number of progeny	Mean spine number	Mean diameter	Number of progeny	Mean spine number	Mean diameter	Spine number	Diameter
Selection (9 days)	76	55	11.16	27.07	21	10.10	23.61	1.06	3.64
Non-selection (13 days)	224	109	12.06	28.09	115	9.94	23.66	2.12	4.43

of determining how large a line could be obtained. These four gave rise to 11 offspring and then stopped reproducing (figure 26, **EDB**). No more offspring appeared, although the specimens were very carefully looked after. These 11 offspring had a mean diameter of 35.54 units and a mean spine number of 17.54. The largest specimen measured 40 units in diameter and possessed 20 spines. This is the largest specimen discovered during the entire course of the work. Arcellas with from 13 to 15 spines and with a diameter of from 34 to 36 units were taken from the pond and many of them reared in the laboratory in other lines, and

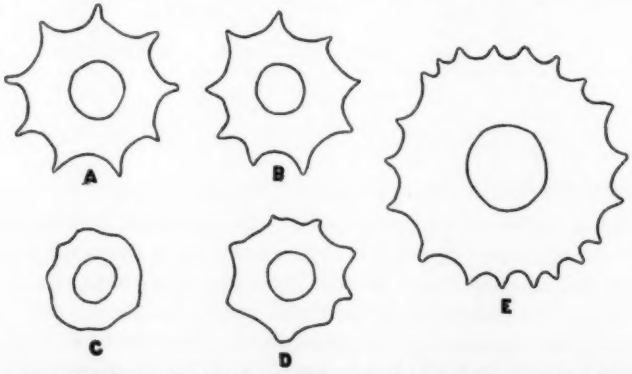

FIGURE 25.—Members of the branch **ED**. **A**, the progenitor of the branch **ED** with 9 spines and a diameter of 112 microns. **B**, the first offspring of **ED**, with 9 spines and a diameter of 99 microns. **C**, the second offspring of **ED**, with indistinct spines and a diameter of 82 microns. **D**, the third offspring of **ED**, with 8 spines and a diameter of 95 microns. **E**, the largest specimen from the subbranch **EDB**, with 20 spines and a diameter of 172 microns. × 207.

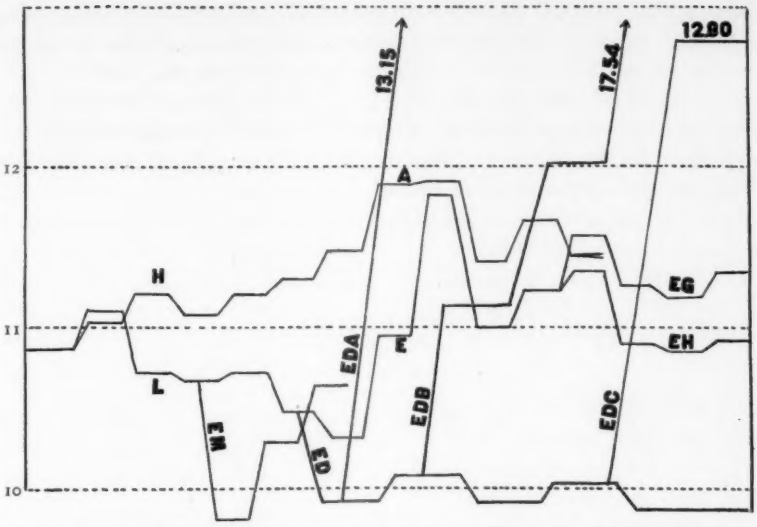

FIGURE 26.—Diagram showing the magnitude of the diversities in spine number that appeared during the vegetative reproduction of family 58. The distances between the horizontal lines indicate the differences between the mean spine numbers of the lines studied. A mean difference of one spine is equal to a vertical distance of 37 mm in the diagram. The diagram shows that before selection was begun, the mean spine number of the members of the family was 10.87. A high line (H) and a low line (L) were obtained during six selection periods with a final difference of 11.48 — 10.32, or 1.16. The high and low lines remained different during four succeeding non-selection periods, the final difference being 11.69 — 11.26, or .43. Selection was then started in the high line and was continued for one period resulting in a difference of only .01 between the high branch **AG** and the low branch **AH**. Selection was also begun in the low line at the same time and was carried on for three periods, **EG** and **EH**, a final difference of 11.21 — 10.87, or .34, being obtained. This was followed by a single non-selection period which ended with a difference of 11.38 — 10.94, or .44.

During the third selection period a very small individual, **EM**, appeared in branch **E**, but this proved to be only a temporary divergence since its descendants by the end of three periods had attained a mean spine number (10.65) at least equal to that of the parent branch **E**.

During the fifth selection period an individual, named **ED**, appeared in branch **E** and produced descendants with a much lower spine number than the parent branch. The main branch **ED** remained at a lower level than the parent branch **E**, but three branches, **EDA**, **EDB** and **EDC** originated from it which reached a higher mean spine number than any other lines studied, namely, 13.15 in branch **EDA**, 17.54 in branch **EDB**, and 12.80 in branch **EDC**. More detailed data will be found in diagram 11 on page 110, and several other branches that were studied will be found described in the text, having been omitted from the diagram for the sake of simplicity.

even specimens with 16 or 17 spines and a diameter of 37 units were recorded, but none were found that approached this in number of spines or in diameter except in this particular branch of the line **ED**.

At the same time that the high line in this branch **ED** was being studied, an attempt was made to obtain a further divergence between the specimens in the low line. Two specimens were selected for the high line and two for the low line as follows:

High line		Low line	
Spine number	Diameter	Spine number	Diameter
10	27	10	24
12	27	9	22

Only five offspring were produced by the two specimens selected for the high line (figure 26, **EDC**). These had a mean diameter of 28.20 units and a mean spine number of 12.80. No further offspring appeared and all of the specimens died.

In the low line after the 2 selected specimens had increased to 22, parents were eliminated and offspring kept whenever reproduction occurred. This procedure was maintained for 42 days (from March 14 to April 24, 1918). Table 30 shows the correlation between diameters and spine

TABLE 30

Family 58. Branch ED. Correlation table for spine number and diameter of shell in low subbranch of the low branch (March 14 to April 24, 1918). The unit of measurement is 4.3 microns. Correlation, .531 ± .041.

Diameter	Spine number 7	8	9	10	11	12	
16	1						1
17							0
18							0
19							0
20			2				2
21			2				2
22	1	1	11	5	1		19
23		1	18	31	9		59
24		1	8	21	13	3	46
25			1		7		8
26					2		2
	1	3	42	57	32	3	139

numbers during this period. The mean spine number of the 139 progeny recorded is 9.88 and the mean diameter of the same progeny is 23.23

(see figure 23). All progeny were below the mean diameter of the line from which their original progenitor was derived and the mean diameter of this branch is nearly the same as the mean of the first large group of 312 reared before selection was begun (figure 23).

It seems clear, therefore, that the line **ED** had become permanently different from the original line **E** from which it arose both with respect to diameter and to spine number. It seems strange that this small derivative of the original line **E** should give rise to three branches **EDA**, **EDB** and **EDC** which are larger than any other branches in the entire family 58 (figure 26). Another phenomenon that needs to be accounted for is the dying out of all of the members of these large branches. The probable reason for this will be pointed out in a later paper, where the relations between nucleus and cytoplasm are described.

Some time after branch **ED** appeared it was discovered in another line that specimens with a single nucleus could be obtained by removing one of the two normally present, and that these uninucleate specimens would produce uninucleate progeny. These uninucleate progeny in every case had shells that were smaller than those of their modified parents as well as fewer spines. All uninucleate progeny that appeared later likewise possessed smaller shells. This discovery led to an examination of the nuclear condition of the members of branch **ED** since the small size of these specimens might be due to the presence of only one nucleus instead of the normal number, two. It was impossible to determine with certainty the nuclear condition of all of the progeny of **ED** because of their small size, the color of the shell, and the large amount of food material often contained in them, but in a large number of cases two nuclei were plainly visible even in some of the smallest specimens measuring 22 units in diameter or less, and only in three or four specimens was it possible to conclude that only one nucleus was present. If the few cases of this kind were thrown out of the foregoing calculations, they would make practically no difference in the results.

EMPTY SHELLS

At irregular intervals during the course of this investigation new shells were formed which separated from the parent organism without being provided with any protoplasm. These empty shells appeared in all the lines under cultivation and no factors were discovered that would account for their production. In family 58 a total of 59 empty shells was recorded or about one percent of the specimens studied in this entire family. Since the empty shells were almost always smaller than their

TABLE 31

Family 58. Table showing the relations between empty shells and their parents and older and younger sisters, with respect to spine number and diameter in ten cases. The unit of measurement is 4.3 microns.

Parent		Empty shell			Older sister		Younger sister	
Spine number	Diameter	Progeny number	Spine number	Diameter	Spine number	Diameter	Spine number	Diameter
11	27	4	8	22	10	25	9	26
12	29	2	9	20	12	28	11	26
10	24	2	8	19	11	24	12	27
9	20	2	7	18	10	22	9	22
9	24	2	8	19	10	25	9	19
11	26	2	10	20	12	27	12	26
11	26	8	8	22	13	27	11	27
8	23	2	9	21	10	24	9	23
8	19	2	7	19	9	20	9	22
9	22	3	7	20	9	22	7	21

parents and possessed a lesser number of spines, they were not included in the data presented in the preceding pages.

Table 31 gives the data with regard to ten representative empty shells. In a few cases the number of spines possessed by the empty shell could not be determined; in a number of cases the diameters of the empty shells, parents, and sisters were not obtained; and in 35 instances the empty shell represented the first offspring and hence had no older sister, and some of the parents were eliminated before a younger sister was produced.

The data obtained show that of the 59 empty shells in family 58,

35 were the first offspring of their parents;
12 were the second;
4 were the third;
3 were the fourth;
3 were the fifth; and
2 were the eighth.

The comparatively large number belonging to the first and second generations is no doubt due to the fact that most of the specimens in the family were eliminated after producing one or 2 offspring. The empty shells possessed fewer spines than their parents in 45 cases, a greater number in 5 cases, and an equal number in 2 cases. They were smaller than their parents in 38 cases; larger in 2 cases, and equal in size in 2 cases.

The relations between the empty shells and their older and younger sisters, i.e., the progeny of the same parent just preceding or succeeding them, were determined with the following results. The spine number of the older sister was greater than that of the empty shell in 18 cases, less in 1 case, and equal to it in 1 case. The spine number of the younger sister was greater than that of the empty shell in 47 cases, less in 2 cases, and equal to it in 5 cases. The spine number of the older sister was greater than that of the younger sister in 6 cases, less in 5 cases, and equal to it in 2 cases. The spine number of the parent was greater than that of the younger sister in 16 cases, less in 21 cases, and equal to it in 5 cases. It seems evident from these results that the production of empty shells has no appreciable influence upon the spine number of later offspring (younger sisters) from the same parent.

Similar relations have been found with regard to the size of the empty shells, their parents, and older and younger sisters. These relations are as follows:

Empty shells were smaller than the parents in 38 cases;
larger in 2 cases;
equal in 3 cases.

Empty shells were smaller than the older sisters in 16 cases;
equal in 1 case.

Empty shells were smaller than the younger sisters in 36 cases;
equal in 2 cases.

Younger sisters were larger than the older sisters in 5 cases;
smaller in 6 cases;
equal in 2 cases.

Younger sisters were larger than parents in 19 cases;
smaller in 12 cases;
equal in 5 cases.

These data show that the empty shells are almost always smaller than their parents, and smaller than their older and younger sisters. The influence of the production of the empty shell upon the succeeding offspring (younger sister) from any given parent is indicated in the last set of data presented above. The next offspring produced after an empty shell is formed is in more than half of the cases larger than the parent. Why this should be so is unknown, but the empty shells are doubtless empty because of a disturbance at the time of reproduction and probably there was not sufficient protoplasm for the production of a normal individual. If this were the case there would be a superabundance of protoplasm ready by the time of the next fission and the next offspring

(younger sister) would tend to be larger than its parent. However, there is no appreciable difference in size between the offspring preceding and the one succeeding the production of the empty shells, so until more extensive data are available the causes and effects of empty shell formation will have to remain unexplained.

It is worthy of note, nevertheless, that two of the sudden increases in diameter and spine number in branch **ED** (see figure 26) were both preceded by the formation of empty shells. In a number of other cases, on the other hand, sudden increases in size and spine number occurred in all the lines under cultivation at the same time without the intervention of empty shell production.

In a number of experiments on Arcellas taken from family 58 or directly from the pond, it was found that specimens when deprived of one of their two nuclei always formed an empty shell just before the binucleate condition was regained. Apparently nothing of this kind happened in the 59 cases described above, since in many specimens the nuclear condition was examined and two nuclei were always present (HEGNER 1919).

Conclusion

We may therefore ignore the empty shells in our studies of diversities in these organisms, at least until we know more about the conditions that cause their formation.

DISCUSSION

A number of questions have suggested themselves during the course of these studies which may profitably be grouped together in this discussion.

1. How does selection operate when practiced on Arcella during vegetative reproduction?

This question is of considerable importance since the significance of the results recorded in this contribution can not be realized until the fundamental difference between selection among sexually and asexually reproducing organisms is clearly understood. If it had been possible to take care of all of the descendants resulting from the vegetative reproduction of the single individual (No. 58) with which these lines were started, and if the pedigree of all these descendants were constructed according to the scheme indicated on page 110, the same lines and branches would appear in this pedigree that have been described in the foregoing pages, but besides this hundreds of other branches would also appear,

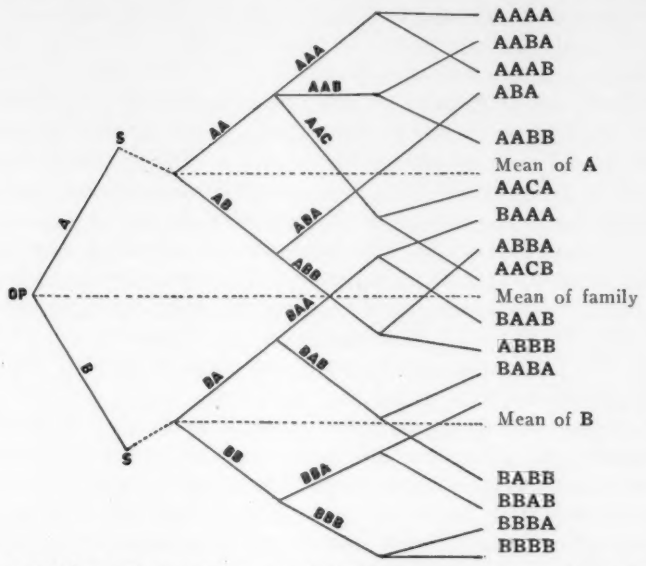

FIGURE 27.—Diagram showing the various heritably diverse branches with respect to spine number and diameter that may arise from a single specimen of Arcella dentata during vegetative reproduction. **OP** = original progenitor. A high line **A** and a low line **B** are isolated by selection. The means of these lines then regress slightly toward the mean of the entire family, but soon become almost stationary although branches from both lines cross each other. For further explanation, see page 145.

such as are indicated in figure 27, branches that were eliminated during the experiments because of lack of time and strength to keep them going. In other words, there has been no selection of existing heritable factors that are contributed to the progeny by two different parents, as is usually the case when selection is practiced among sexually reproducing organisms; but specimens have been picked out from a pedigree resulting from the successive divisions of a single germ plasm.

2. Are shell characteristics influenced by the environment?

There is a peculiarity exhibited by many of the correlation tables that have been made up that needs consideration. Offspring of parents with a spine number near the lower limit, e.g., those of parents with 8 or 9 spines, possess an exceptionally high mean spine number.

Two sets of factors must be considered in interpreting such tables, (1) hereditary factors and (2) environmental factors. It seems perfectly clear that the influence of heredity is very strong. As regards the

environment, although this was kept as uniform as possible at all times, still small solid objects such as minute grains of sand might modify the shell of the offspring while it is still in a plastic condition, so as to prevent the development of the complete (hereditary) spine number. This would account for *some* of the offspring with 7, 8 and 9 spines that were produced by parents with 11 and 12 spines. The hereditary factor would tend to express itself again in the next generation, hence, e.g., offspring with 8 spines would produce offspring with 10, 11, 12 or 13, as shown in the tables. This may account for the exceptionally high mean spine number of the progeny of parents with 8 spines as shown in table 5. Many of the low-spined specimens here recorded were not regular, but, as indicated in figure 12, **C**, large spaces sometimes occurred which might be due to the suppression of one or more spines by some obstruction in the environment.

Mechanical environmental factors might thus cause a decrease in spine number, but probably could not produce an increase; thus the mean spine number of the offspring of high-spined parents would tend to be lower than their hereditary constitution. Other environmental factors may affect the organism at the time of reproduction or during the intervals between fissions. In the latter case the results of their influence would not be visible until reproduction occurs, and then only in the offspring, since the shell of the parent is not modified after it is once formed.

In this connection we may also refer to the increases and decreases in spine number that have several times been found to occur simultaneously in all of the lines under cultivation. Examples of this are cited in both the high and low lines of family 58 (page 121) and in branch **EM** (page 131), and data are presented in tables 17 and 27. The most plausible suggestion to account for this is that some cultural condition favorable for the growth of the organisms caused an increase in size, and a subsequent less favorable condition resulted in a reversion to the previous state which we have called "normal." This increase in size would be accompanied by an increase in the spine number, since these two characters are closely correlated.

3. Why was there a decrease in the amount of diversity between the high and low lines of family 58 after selection was discontinued?

The lesser difference between the high and low lines at the end of the non-selection periods than at the beginning is probably due to the appearance in the high line of heritable variations toward a lower spine number, and in the low line of similar variations toward a higher spine num-

ber. Specimens exhibiting such variations would have been removed during the selection periods and hence would not have been allowed to produce offspring which would lower or raise the means in the two lines.

In other words, the differences between the means of the two lines during the selection periods appear greater than they really were, and when selection was stopped, the true condition was revealed. From this time on there should be no great fluctuations in the magnitude of the difference between the means except those due to environmental influences. This proved to be true, as the data show clearly.

Figure 27 was made on the basis of the data presented in this paper to illustrate what probably happens during the vegetative reproduction of *Arcella dentata*. Part of the hereditarily diverse branches that result during the vegetative reproduction of a single specimen are here shown. Suppose for purposes of illustration that we have by selection picked out of the entire pedigree the high line **A** and the low line **B**. If we then discontinued selection we would obtain a pedigree in which lines **A** and **B** would give rise to the branches **AA**, **AB** and **BA**, **BB**, and from these in turn would be derived various other branches, a few of which are indicated in the diagram. Some of these branches would have a mean similar to **A** and **B**, but other branches would have a mean either above or below that of the line from which they sprang. Finally an upper limit, represented in the diagram by branch **AAAA**, would be reached beyond which an increase in size and spine number is not possible, just as there is a well known limit to the size of all species of organisms. And similarly a lower limit, **BBBB**, would also be reached. Some of the branches of the high line **A**, such as **ABBB**, would coincide with or even possess a lower mean size and spine number than certain branches of the low line **B**, such as **BAAB**. And certain branches of the low line **B**, such as **BAAA**, would become higher than some of the branches of the high line **A**, such as **AACB**. The mean size and spine number of the entire group of specimens obtained after selection ceased (**S** in figure 27) would at first decrease slightly in line **A** and increase slightly in line **B**, but thereafter would fluctuate very little and the two lines **A** and **B** would remain distinct indefinitely.

The fact that there appears to be both an upper and a lower limit to the size and spine number in these organisms may account for the slight difference of .07 during the second non-selection period (see figures 11 and 26). When the high line reached a mean spine number of 11.90 during the first non-selection period, its upper limit for this character had about been reached. After this upper limit had been reached by the high

line, the low line continued to increase in spine number until it almost attained this upper limit, having a mean spine number of 11.84 in the second non-selection period. Then when the environment changed and a decline in spine number set in, the mean of the low line decreased more than that of the high line and a difference of .41 resulted during the third non-selection period. A simultaneous increase in spine number again appeared during the fourth non-selection period but at this time there was no near approach to the upper limit and the increase in the high line (.27) was greater than that in the low line (.25).

5. Are diversities due to large or small heritable variations?

The studies of family 58 suggest that the diversities obtained were due to barely distinguishable heritable variations and that an actual shifting of the mean and the mode has occurred in this way. The establishment of branch **ED**, however, as a permanently small line, proves that hereditarily diverse lines may be produced by the appearance of a few extreme specimens. The fact should not be overlooked that when an extreme specimen arises, such as **EM** (page 129), whose diversity is not hereditary, it requires several generations before the mean condition of the line is regained. It may therefore also be true that a large change in the hereditary constitution of a specimen would not appear in its entirety in the first generation, although the f_1 progeny would exhibit it in part, but would become fully manifest only in about the third or fourth generations. While it does not seem probable that the high and low lines of family 58 have originated in this way, still it is a possibility that must be kept in mind.

It also seems probable that mutations may have occurred more often than actually observed, since, because they were not visible in the first generation, they might have been discarded. Especially is this true of mutations toward a lower spine number in the high line and a higher spine number in the low line. This may account for the fact that the supposed mutations discovered in the latter were all smaller than the mean of the line.

6. How do diversities in heritable characteristics originate during vegetative reproduction in *Arcella dentata*, i.e., what is the method of evolution in these organisms?

The germ plasm of organisms that are producing vegetatively is commonly supposed to divide into two qualitatively equal parts at each division and the descendants therefore should all be alike. The fact that branches of such a family as No. 58 are permanently diverse, proves that changes have occurred in the *constitution* of the germ plasm during vegetative reproduction; that is, evolution has taken place. These changes in

the constitution of the germ plasm may be due to two causes, (1) unequal distributions of qualitatively different germinal materials (factors or parts of factors?) during fission, or (2) actual changes (chemical?) in the germ plasm arising spontaneously or initiated by the internal or external environment.

The presence of a conspicuous network of chromidia in Arcella, which has been identified as idioplasm, may lead during fission to differences in parent and offspring in chromatin content which would account for the heritable diversities discovered. The removal of part of this chromidial network, however, as will be shown in another paper, has no effect upon the heritable characteristics of the line. It may also be that nuclear reorganization processes occur during vegetative reproduction, such as take place in Paramecium, but nothing of the kind has been observed in the thousands of specimens examined, although the nuclei may easily be seen in the living animal. This problem can not profitably be discussed further at this time, since, although more is known about the nuclear phenomena and reproductive processes in Arcella than in any other rhizopod, still many of the investigations need to be checked up and there is much still to be learned. Arcella, however, offers many advantages for the study of the problem of the method of evolution and further investigations along this line are now in progress.

7. How do the results obtained from these studies of *Arcella dentata* compare with those previously reported by other investigators?

Most of the investigators who have attempted to change the genotype by selection in Protozoa that are reproducing vegetatively, have failed.[2] MIDDLETON (1915), however, succeeded in obtaining from a single progenitor two lines of Stylonychia that differed in fission rate, and JENNINGS (1916) demonstrated the fact that vegetative reproduction in *Difflugia corona* is accompanied by minute heritable variations in a number of measurable characters resulting in branches that differ markedly in their genotypic condition. The investigations reported in this paper show that *Arcella dentata* resembles *Difflugia corona* in this respect and confirm many of JENNINGS's discoveries. Since these two organisms resemble each other very closely, it may be worth while to compare briefly the results of JENNINGS's investigations on Difflugia with those reported herein on Arcella.

Among the characters of *Difflugia corona* studied by JENNINGS are spine number and diameter. The spines of *Difflugia corona* vary from

[2] For the literature on this subject see JOHANNSEN (1913), MIDDLETON (1915), JENNINGS (1916), ACKERT (1916), ROOT (1918).

0 to 14, those of *Arcella dentata* from 7 to 20. In both organisms, (1) families resulting from the vegetative reproduction of single "wild" specimens were obtained which were hereditarily diverse with regard to spine number; (2) families diverse in shell diameter were discovered; (3) deviations of the parents from the mean spine number and diameter are in part inherited by the offspring; (4) diameter and spine number are closely correlated, and the greater the diameter the more numerous are the spines; and (5) the long-continued selection of the progeny of a single specimen that shows deviations in these characters results in the isolation of lines that are heritably diverse. Mutations occur in both Difflugia and Arcella, but diversities in lines derived from a single specimen seem to be due rather to the accumulation of many small heritable variations than to large ones.

SUMMARY

1. The main problem attacked in this investigation is, Can heritably diverse lines be recognized among the descendants of a single specimen of *Arcella dentata* produced by vegetative reproduction?

2. *Arcella dentata* was chosen as material because it possesses several definite measurable characters, especially the number of spines and diameter of the shell, which are fully determined at the time of reproduction and are not thereafter affected by growth changes, or by environmental factors (figure 1).

3. "Wild" specimens vary in spine number from 7 to 17 and in diameter from 73 microns to 150 microns; these characters are correlated, and on the average the greater the diameter, the more numerous are the spines. (Tables 1 and 2.)

4. Variations in spine number occur among the descendants of a single specimen produced by vegetative reproduction, and these variations are in part inherited.

5. The hereditary constitution of different families obtained by vegetative reproduction from different "wild" specimens is different with respect to spine number and diameter. A "wild" population therefore consists of a large number of hereditarily diverse families that may be isolated in the laboratory. (Tables 3, 4 and 5.)

6. A single large family (No. 58), containing 5557 specimens and representing 69 generations, was obtained from a single specimen by vegetative reproduction. Selection within this family led to the isolation of hereditarily diverse branches as follows (figures 11 and 26).

(a) Using mostly past performance as a basis for selection, two lines

were obtained during a period of 64 days and involving the study of 1192 specimens, representing 22 generations. The difference in spine number between these two branches at the end of this period was 1.16 and the mean difference for the entire period was .55. A difference in spine number was maintained during a succeeding non-selection period of 35 days; 1325 specimens and 18 generations were studied during this period. The difference in spine number between the high and low lines persisted throughout the non-selection period, but decreased to an average mean of .46. This was to be expected since hereditary diversities are constantly appearing both toward an increase and a decrease in spine number (see page 145). (Tables 6 and 14.)

(b) The low line thus obtained was subjected to a period of selection of 23 days during which 722 specimens were obtained, representing 15 generations. Two hereditarily diverse branches of this low line appeared with a mean difference in spine number of .30 which increased during a short succeeding non-selection period. (Table 19.)

(c) Measurements of the diameters of the low and high lines mentioned in (a) show that these lines also were diverse with respect to the diameter of the shell and that spine number and diameter are correlated throughout the entire family. The mean difference in diameter between the high and low lines was .34 units.[3] (Tables 21 and 22.)

(d) Similarly there was a mean difference of .26 units in diameter between the high and low branches of the low lines. (Table 23.)

(e) Four branches of family 58 were studied whose progenitors were markedly smaller and possessed a lesser number of spines than the other specimens in the low line in which they appeared. Three of these branches reverted to the mean condition of the low line within a few generations, but one of them, **ED**, proved to be permanently diverse in both spine number and diameter. It differed from the mean of the low line by 1.08 in spine number and by 3.54 units in diameter. (Figure 23.)

(f) From this branch **ED** there appeared at different times three distinct branches (**EDA**, **EDB**, and **EDC**) containing specimens larger and with more numerous spines than those encountered in any other part of the entire family 58. (Compare figures 13 and 25.)

7. Empty shells are often produced by apparently normal specimens. They are almost always smaller than their parent, and smaller than their older and younger sisters. Their appearance seemed to have no influence upon the heritable diversities studied. (Table 31.)

[3] The unit of measurement is 4.3 microns.

8. A large family of *Arcella dentata,* therefore, derived from a single specimen by vegetative reproduction, consists of a number of branches that are hereditarily diverse with respect to diameter and spine number. These diverse branches resemble the hereditarily diverse families that were obtained by vegetative reproduction from different "wild" specimens.

9. The formation of such hereditarily diverse branches appears to be a true case of evolution that has been observed in the laboratory and that occurs in a similar way in nature.

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VARIATION AND SELECTION WITHIN CLONAL LINES OF *LEMNA MINOR*[1]

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INTRODUCTION

The experiments herein reported were conducted for the purpose of adding to our knowledge concerning clonal variation and the effect of selection on such variation, in relation to the pure-line concept. It is unnecessary here to include a review of published data. It is, of course, generally recognized that the greater bulk of experimental evidence supports the pure-line theory as elaborated by JOHANNSEN.

MATERIAL USED

The plants used in these experiments are commonly called duckweed

[1] Paper No. 73, Department of plant breeding, CORNELL UNIVERSITY, Ithaca, N. Y.

and belong to the genus Lemna. This, according to GRAY (1908), is a genus widely distributed over Europe, Northern Asia and North America, but rare in the Tropics. The duckweeds are small, floating plants without distinct stems or real leaves, and may or may not have roots. They rarely produce flowers, the usual mode of propagation being through budding. The present paper is concerned only with one species, *Lemna minor* Linn.

It is necessary to give a more than passing statement regarding the mode of budding. The main structure of the plant is usually called a frond. Some botanists regard it either as a stem, or leaf, or both fused together. The term "frond" is used throughout this paper. According to BLODGETT (1915) the frond consists of three parts: (a) a terminal leaf, (b) a bud rudiment inclosed by a flattened bud scale and (c) an apical region from which new fronds are developed. Vertical pressure during the early stages of growth causes the splitting of the bud rudiment into two buds which do not develop at the same time. These outgrowths come out as a horizontal series in an overlapping form through the lack of space for vertical succession. The development of the basal region into a stalk or stipe causes the thrusting forward of each new whole structure. In *L. minor* this basal region is attached marginally to the main portion of the frond; in other species, as in *L. polyrrhiza*, it is inserted upon the vertical surface some distance from the edge. Figure 1 shows a parent frond with its offspring still attached to it. The members of the family are numbered consecutively in the order of the time of their appearance.

VARIATION WITHIN A WILD POPULATION

Before studying clonal variations a study within a wild population was made concerning shape and size of fronds, speed of propagation and root habits.

Shape of frond

Figure 2 shows fronds of various shapes taken from a population which was collected on December 10, 1916, from a stagnant creek at the Ithaca fair-grounds. The sketches were made by examining the specimens under Zeiss binoculars and tracing the outlines of the image as thrown over the paper with the aid of a Zeiss camera lucida. In all cases mature fronds, such as had already turned yellow but which were still attached to their offspring were studied, thus eliminating, as far as possible, the effect of different ages. From the figure just referred to it

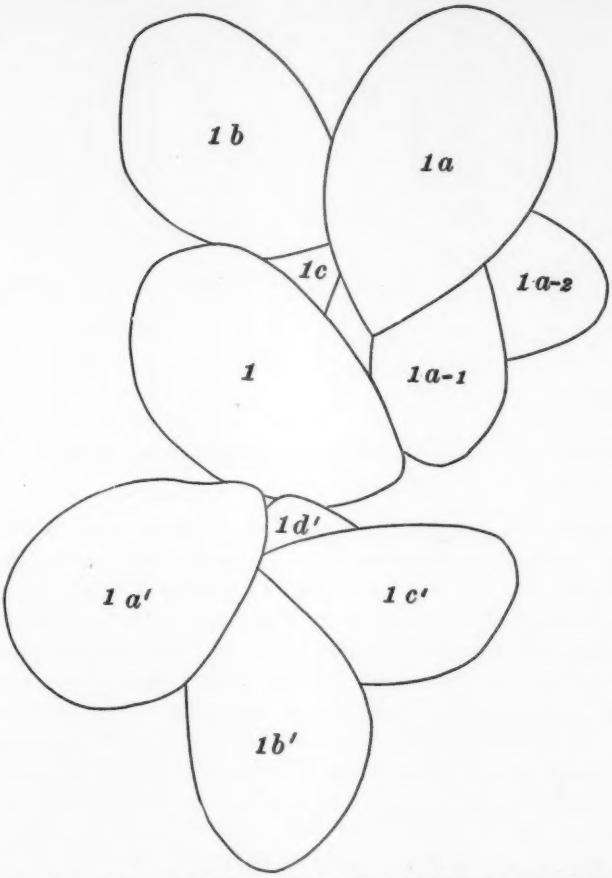

FIGURE 1.—A parent frond with its offspring still attached to it. × 16 diameters.

may be seen that there exist diverse forms of fronds in a wild population. To determine whether or not differences in shape are inherited, that is, to ascertain if different forms represent distinct strains, several fronds were isolated from the wild stock. Each frond was allowed to propagate in a tumbler containing tap water and kept in a greenhouse section in which the temperature was generally 15° C at night and 25° C by day. Preliminary cultural experiments had shown that the plants die after a time if frequent change of water in the culture tumblers

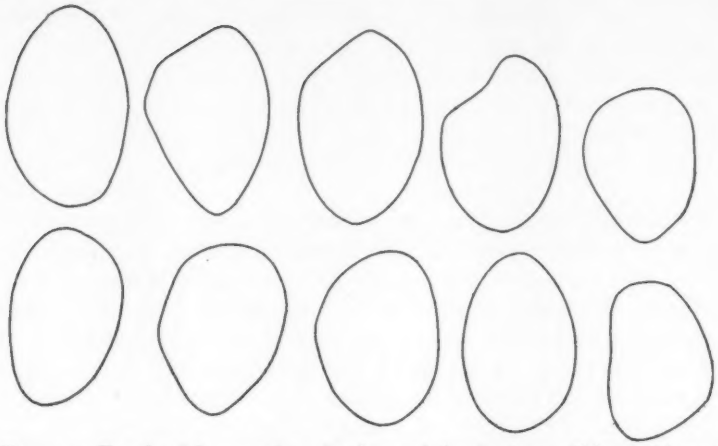

FIGURE 2.—Fronds of *Lemna minor* showing variation in shape within a wild population. × 16 diameters.

is not made. To meet this difficulty, the tumblers were arranged in rows, the members of each row being connected with one another with siphon tubes. By allowing the water to siphon from a big deposit jar into the tumblers at the head of the rows, this water in turn being siphoned into those that follow, a provision was thereby made which permitted a partial but continuous change of water most of the time.

Figure 3 shows camera drawings of fronds from two clones. Each figure shows individuals from one line. From a close study of these and similar unpublished drawings it was seen that while the individuals within a line vary in shape to a greater or less degree, there is much more resemblance among members of the same clone than among those of different lines. It is only fair to conclude from this that in a wild population there exist races of diverse shape.

Speed of budding

The term "speed" does not imply "rate." There is no use of studying variation in rate of budding in *L. minor* since different fronds have the same rate of budding. Each frond produces invariably two buds and no case has yet been reported where more or less than this number has been produced. However, different fronds may require different lengths of time to produce their offspring buds. Speed of budding may be measured either by noting the number of days it takes for a given number

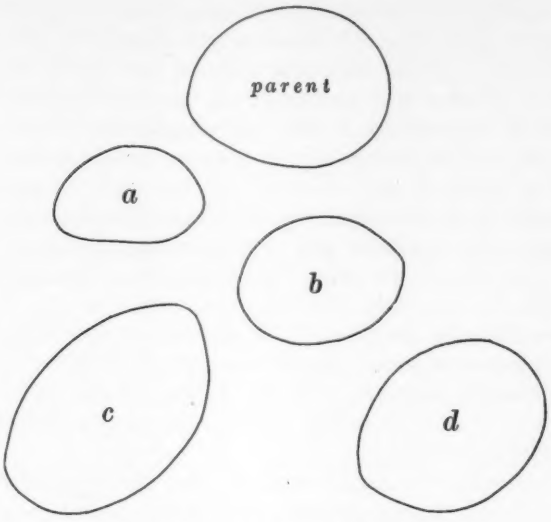

FIGURE 3.—Fronds from clones 18 and 26. × 16 diameters.

of new individuals to be produced from an original frond, or by determining the number of individuals produced within a given length of time. The latter method is simpler and was used in this study.

In this experiment it is necessary that the starting fronds be of the same age. In this and in all other cases where there was necessity of using individuals of the same age, a number of fronds from which no bud had yet appeared were selected from the stock. These were then observed and all fronds appearing for the first time on the same day were taken to be of similar age. By increasing the initial number of starting fronds almost any reasonable number of similar-aged buds could be obtained.

To determine the variation in the speed of propagation, each of a number of buds of the same age from which the first buds appeared at the same time was placed in a culture tumbler and there allowed to propagate. After a certain number of days, the total number of fronds in each tumbler was counted.

Table 1 contains the results obtained from three determinations and gives a rough idea of the degree of variation in the speed of reproduction.

TABLE 1
Variation in speed of reproduction.

Class values	Frequency		
	Dec. 30-Jan. 9	Feb. 20-Mar. 2	Feb. 25-Mar. 7
3	0	0	0
4	5	2	3
5	8	8	3
6	14	8	15
7	22	26	30
8	7	10	4
9	3	5	2
10	1	1	3
Mean	6.517 ± .115	6.883 ± .110	6.783 ± .107
σ	1.323 ± .081	1.266 ± .078	1.266 ± .075
C. V.	20.30 ± 1.30	18.39 ± 1.16	18.07 ± 1.14

The variations shown in the preceding table do not appear to be multimodal and do not indicate that they represent different speed strains.

Variation in the habit of root growth

It is commonly observed that there is a tendency for plants of *L. minor* to produce curly or twisted roots. The manner of this curling or twist-

ing is by no means uniform. While in general the curling is only immediately below the tip, other plants have longer portions of their roots in a twisted condition. In a few cases, the twisting may even come to the middle of the root.

The value of this habit of the plant as a character for the study of variation depends upon whether it is hereditary or is merely the effect of environment.

Unfortunately, variation in this character cannot be measured with any degree of accuracy and does not lend itself readily to genetical study. What is worse, it makes the study of the variation in size, such as in length of the roots almost impossible. An attempt was made to grow a number of the duckweeds on 2 percent agar-containing nutrient solution, hoping to get straight roots which would lend themselves to measurements, but this attempt failed, the roots refusing to grow or sink into the agar media.

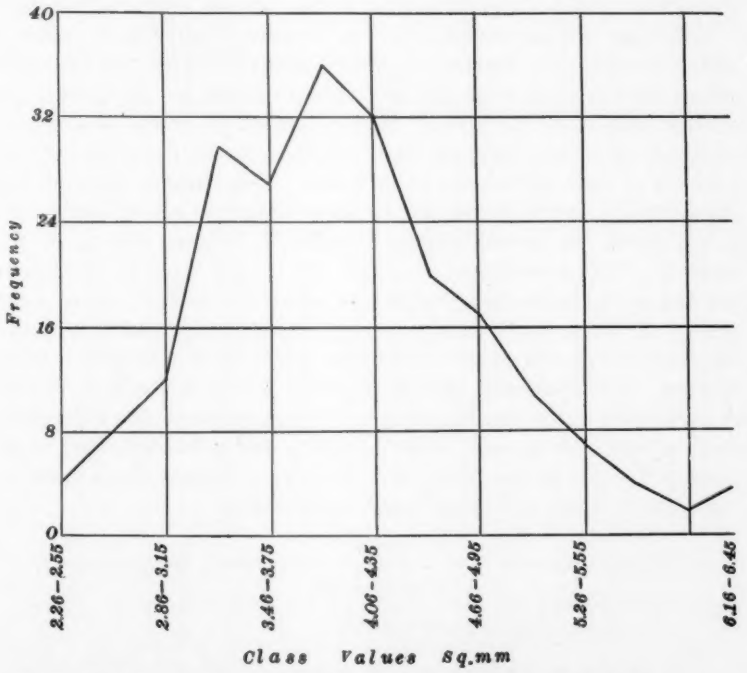

FIGURE 4.—Curve showing variation in size in a population of *L. minor*.

Variation in size of fronds

The size of a frond was determined by measuring its camera-magnified area with the aid of a small planimeter and then computing the true area by dividing the magnified area by 256, the number of times the object was magnified. Two hundred mature-population fronds which were of the same age and which matured at the same time were so measured. Table 2 gives the results of the measurement and figure 4 shows the frequency curve. The curve shows a tendency to three modes, one of these occurring at 3.16-3.45 mm², another at 3.76-4.05 mm², and a third at 6.16-6.45 mm². It might be concluded from this that in a population of *L. minor* there is a probability of the existence of diverse size strains. Such diverse strains need not be found in all localities since the extreme rareness with which this plant has a chance to cross-breed and the rapidity with which it reproduces by budding, both tend, with the help of natural selection, to reduce the inhabitants of a locality to that of a clonal line. None of the clonal lines studied showed a bimodal condition.

Following the determination of the frequency distribution shown in table 2 it would have been only logical to ascertain whether the size modes persist, that is, whether or not the size races found are permanent. An attempt was made to do this. It was planned to isolate several lines representing widely different sizes and then to determine at different intervals of time the average of each line. This attempt, however, was unsuccessful. It was found that *L. minor* cannot be grown successfully in tap water for several months in spite of frequent change of this medium. After a month or so, the fronds usually begin to decrease in size and by the time when enough individuals are needed to give a fair sample, the lines usually have run out. As will be learned later in this paper, continuous culture was maintained by the use of a mineral nutrient solution. It was deemed unwise, however, to use this culture in such an experiment as the determination of the persistence of size differences, since, as will soon be seen, mineral solution had a decided effect in increasing the size of the fronds and no form of culture check could be devised with which this effect could be controlled.

TABLE 2

Distribution of variation in the size of 200 fronds from a wild population of Lemna minor.

	Class values in square millimeters													Mean	σ	C. V.
	2.26-2.55	2.56-2.85	2.86-3.15	3.16-3.45	3.46-3.75	3.76-4.05	4.06-4.35	4.36-4.65	4.66-4.95	4.96-5.25	5.26-5.55	5.56-5.85	5.86-6.15	6.16-6.45			
Frequency	3	7	11	29	26	35	31	19	16	10	6	3	1	3	4.019±.037	.767±.026	16.60±.570

VARIATION AND SELECTION IN CLONAL LINES

Variation and selection in shape of frond

It has been seen already, in the discussion of the permanence of shape strains, that different clones with distinctly different-shaped fronds tend to reproduce their respective characteristic shapes. A certain amount of variation in shape within the clones was also pointed out. Further studies along this line were carried out. The plants, as previously, were grown in tumblers, but in mineral nutrient solution instead of tap water. The use of this solution made the continuous change of culture media unnecessary. The nutrient solution was prepared according to the following modified formula of PFEFFER:

Constituents	Grams per liter
$Ca(NO_3)_2$	0.4
NaCl	0.1
$MgSO_4$	0.1
KH_2PO_4	0.1
$Fe_3(PO_4)_2$	0.1
KNO_3	0.1

To study the variation in shape, one hundred mature fronds grown

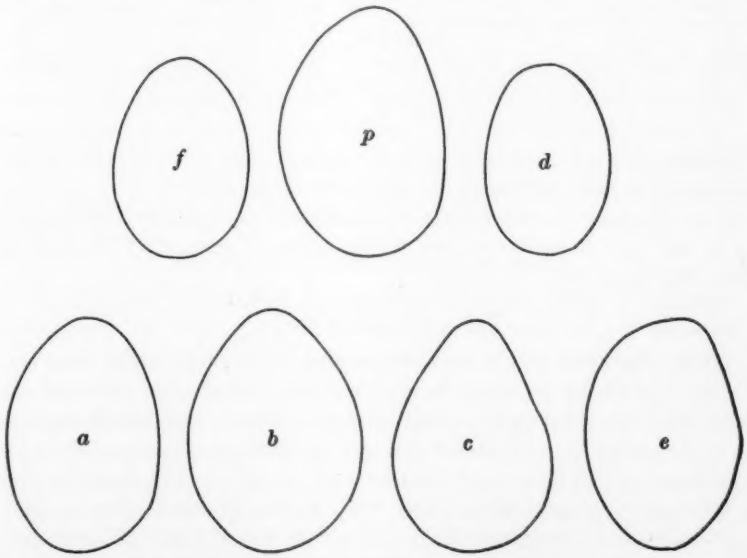

FIGURE 5.—Variation in frond shape in clone 35. × 16 diameters.

from buds of the same age were drawn for each clone. The resulting drawings were classified according to shapes. Figure 5 will give some idea of the dominant shape and the shapes of the varying individuals in clone 35. This dominant shape is represented by the letter p, while the varying shapes are represented by letters a, b, etc.

The frequency of the different shape types in four clones studied is given in table 3.

TABLE 3
Frequency of shape variants.

Clone	Shape types							Total number of individuals
	p	a	b	c	d	e	f	
35	51	3	22	17	1	2	4	100
	Shape types							
	p	g	h	i	j	k	l	
38	47	21	13	11	5	2	1	100
	Shape types							
	p	m	n	o	q	r		
76	48	7	10	15	18	2		100
	Shape types							
	p	s	t	u	v	w		
81	46	14	13	13	1	13		100

From table 3 it may be seen that in clonal lines there exist different shapes of fronds, with some one type predominating.

Before taking up the subject of inheritance in shape in clonal lines, it is well to discuss the results of the study of several of the factors affecting variation.

Effect of culture media

Before this part of the experiment was undertaken, it had been observed that fronds growing in tap water had a decidedly different appearance from those growing in nutrient solution. This was partly due to a difference in size; those growing in nutrient medium were very much larger than those in tap water. Suspecting that there may be also a difference in general shape in the two cultures, it was decided to carry out experiments along this line. Clones 38, 39, 41 and 79 were used. Parallel cultures were set up for each clone. Initial buds of those grown

in tap water came from stock growing in tumblers containing water and garden soil, while buds of those grown in nutrient solution came from stocks already growing in nutrient medium. The four series were not grown at the same time as were the paired cultures from each clone. From each culture one hundred mature fronds were harvested, drawn, and classified according to shape. Table 4 gives the frequency of the different types observed.

TABLE 4

Frequency of shape types of plants grown in tap water and in nutrient solution.

Clone 38			Clone 39			Clone 41			Clone 79		
Types	Tap water	Nutrient solution	Types	Tap water	Nutrient solution	Types	Tap water	Nutrient solution	Types	Tap water	Nutrient solution
a	18	28	i	24	36	o	0	8	u	27	41
b	20	3	j	16	18	p	49	12	v	15	17
c	7	12	k	0	17	q	28	54	w	10	10
d	2	1	l	12	4	r	16	11	x	0	1
e	33	7	m	8	13	s	7	9	y	48	20
f	0	1	n	40	12	t	0	6	z	0	11
g	0	3									
h	20	45									
Total number	100	100		100	100		100	100		100	100

Table 4 shows two important points: (1) in every case there was found greater variation in nutrient-grown plants than in those grown in tap water, and (2) the predominant shape in each clone is different for the two culture media. In clone 38, for example, shape e was predominant among the tap-water-grown plants while among those grown in nutrient solution shape h was the predominating type.

Inheritance of shape within a clone

It has already been pointed out (see table 3) that a study of one hundred mature fronds of clone 81 revealed six shape types, s to w, with type p predominating. To determine to what extent these different shape types are hereditary, a family was bred from each type in nutrient solution and one hundred mature fronds from each were drawn and studied as to variation in shape. Table 5 contains the results of this study.

We see from table 5 that the parental type seems to have had no effect on the type distribution (excepting the type representing the clone. An interesting fact brought out by the above data is that while the diverse shapes which do not represent that of the clone were not hereditary, they

TABLE 5
Frequency of types in different families of clone 81.

Parent types	Types of progeny and their distribution										
	s	t	p	u	v	w	g	h	i	j	k
s	1	7	43	13	1	17	0	6	2	6	4
t	0	6	36	17	1	9	7	12	1	5	6
p	3	8	51	9	1	9	5	5	1	6	2
u	1	6	47	3	0	30	0	4	1	8	0
v	1	6	47	9	2	19	3	8	1	3	1
w	2	8	41	7	1	25	2	10	1	3	0

appeared in approximately the same relative proportion to one another irrespective of their parental shapes.

In order that this point may be seen more clearly, the data in table 5 were made into curves shown in figure 6.

Another attempt to change the dominant shape type of clone 81 was made by continuous selection of shapes u and w. The experiment was carried through three periods, each period comprising many generations. There were three cultures during each period, one for u selection, one for w and another for p. The latter served as control. One hundred mature fronds were examined from each harvest. Table 6 contains the results.

TABLE 6
Results of continuous selection for types u and w in clone 81.

Parent shapes	Shapes of progeny and distribution										
	s	t	p	u	v	w	g	h	i	j	k
	First period										
u	1	5	49	10	2	25	1	2	2	3	0
p	3	5	48	11	2	21	1	2	2	5	0
w	7	3	46	4	2	20	2	7	2	7	0
	Second period										
u	6	12	38	5	0	27	0	10	2	0	0
p	3	13	35	5	1	31	3	8	1	0	0
w	10	17	26	4	2	22	6	7	6	0	0
	Third period										
u	17	0	50	12	5	8	3	2	3	0	0
p	13	7	37	18	3	22	0	0	0	0	0
w	7	5	29	16	7	17	8	7	4	0	0

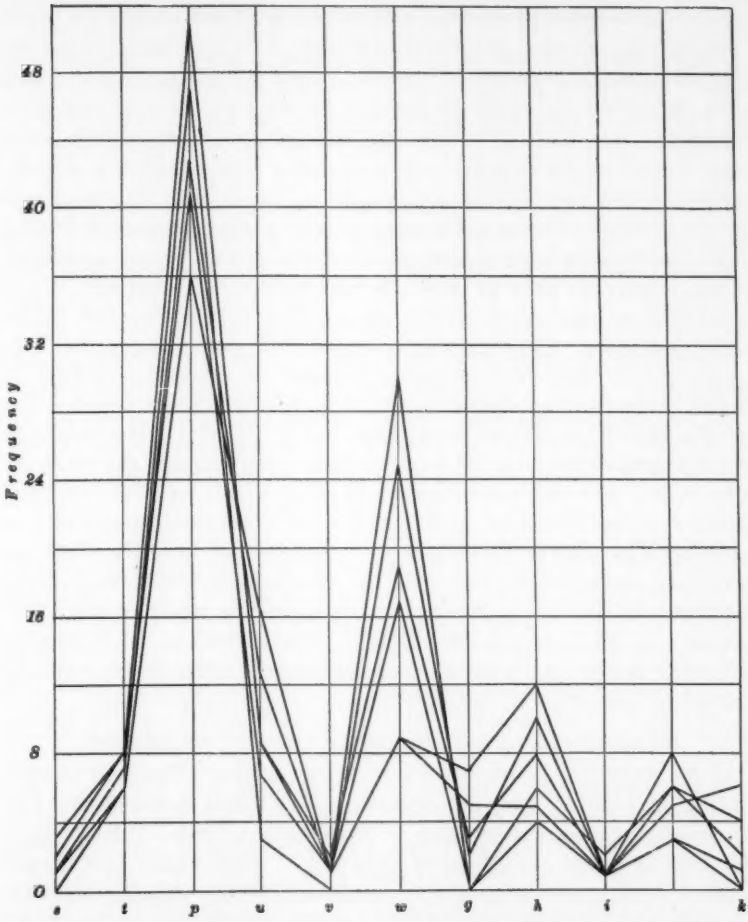

FIGURE 6.—Frequency curves of different shape types in different families of clone 81.

It would appear from the results shown in tables 5 and 6 that the different non-dominant shape types in clone 81 are merely somatic variations, probably physiological, and are not inheritable, and that selection for these different shapes has made no progress. As early as 1894, GUPPY (1894) reported that long exposure to different habits of life, as growing in mud, had not produced any permanent change in the external appearance of duckweeds.

Unusual non-heritable variations in frond shape

During the entire period of investigation, a watch was continually kept for mutations. Three fronds of unusual shapes appeared in nutrient cultures, two in clone 41 and one in clone 42. When they were found, they were still attached to their parent fronds. Each of these unusual-shaped fronds together with each parent was placed in a separate culture tumbler and allowed to propagate to determine if they were mutations. When matured individuals in each tumbler numbered fifty or more, the cultures were discontinued and the mature fronds examined. It was found that none of these aberrant shapes was hereditary.

Selection in opposite directions was made in each of the four clones mentioned above. Each selection was carried through five periods. Plus selection was made by continuous selection of individuals falling in classes 9 and 10, and minus selection, of those in classes 5 and 4. A check culture of unselected individuals was also grown. The three cultures in each clone—plus, minus and check,—were always grown at the same time. The plants were grown in the nutrient medium and good care was taken to render cultural and other controllable conditions as much alike as possible for each series. Tables 8-11, inclusive, show the results of this selection, and table 12 contains the differences between the means of the check cultures and those of the plus and minus selections. If the selection be effective, there should be an increasing difference between the means of the check and selection series from the first to the last period of the experiment.

Clonal variation and selection in speed of propagation

In this study, clones 38, 39, 78 and 81 were used. The unit of time taken was 11 days. Sixty individuals were studied in each culture. Initial studies of variation in speed of propagation of these different lines gave results which are shown in table 7. A "class value" in this case represents the total number of individuals obtained by allowing an original bud and its offspring to propagate during 11 days.

TABLE 7
Clonal variation in speed of propagation.

Clone	Period	Class values									Mean	σ	C.V.
		3	4	5	6	7	8	9	10	11			
38	May 18-June 18	0	3	6	8	23	11	8	1	0	7.017±.118	1.360±.084	19.38±1.24
39	May 18-June 18	1	2	2	6	24	11	8	5	1	7.433±.133	1.532±.094	20.61±1.32
78	June 11-June 21	0	3	7	11	25	10	3	1	0	6.750±.109	1.247±.077	18.47±1.17
81	June 19-June 29	0	2	3	12	27	10	4	2	0	7.000±.104	1.197±.074	17.10±1.08

From table 7 it is to be seen that clones 38 and 81 seem to have the same speed of budding. The other two clones, however, appear to possess distinctly different means, whose difference is .683±.172, so that it may be considered as highly probable that in a population of *Lemna minor,* there exist also different strains in regard to speed of asexual propagation.

TABLE 8

Selection in speed of propagation in clone 38.

Selection	Frequency of class values									Mean	σ	C.V.
	3	4	5	6	7	8	9	10	11			
First period												
Plus (+)		2	7	15	26	8	2			6.617±.093	1.066±.066	16.11±1.02
Check		1	7	14	27	9	2			6.700±.082	1.021±.063	15.24±0.96
Minus (—)		1	10	12	25	10	2			6.650±.095	1.093±.067	16.44±1.04
Second period												
Plus (+)			8	15	28	6	3			6.683±.086	0.991±.061	18.83±0.93
Check			7	15	27	8	3			6.750±.087	0.994±.061	14.72±0.92
Minus (—)		3	8	17	23	6	3			6.500±.100	1.147±.071	17.65±1.12
Third period												
Plus (+)		3	8	13	26	8	2			6.567±.098	1.131±.070	17.22±1.09
Check		1	11	15	26	5	2			6.483±.091	1.041±.064	16.06±1.01
Minus (—)		3	6	14	25	10	2			6.650±.098	1.123±.069	16.89±1.07
Fourth period												
Plus (+)		3	4	10	25	12	6			6.950±.106	1.217±.075	17.52±1.11
Check		3	8	10	25	11	2	1		6.717±.108	1.240±.076	18.46±1.17
Minus (—)		2	4	11	24	11	5	3		7.083±.114	1.308±.081	18.47±1.17
Fifth period												
Plus (+)		2	8	12	26	9	2	1		6.700±.102	1.173±.072	17.51±1.11
Check		1	10	14	25	7	3			6.600±.096	1.098±.068	16.64±1.05
Minus (—)		3	9	13	24	9	2			6.550±.101	1.161±.071	17.72±1.12

TABLE 9

Results of continuous selection in speed of propagation in clone 39.

Selection	Frequency of class values										Mean	σ	C. V.
	3	4	5	6	7	8	9	10	11	12			
First period													
Plus (+)		3	3	7	30	8	6	3			7.117±.115	1.318±.081	18.52±1.18
Check		2	4	4	30	8	8	3	1		7.317±.121	1.384±.085	18.91±1.20
Minus (—)		2	6	6	29	10	4	2	1		7.067±.117	1.340±.083	18.96±1.21
Second period													
Plus (+)	1	4	6	28	11	9	1				6.250±.103	1.178±.073	18.85±1.20
Check	2	2	5	29	9	7	5	1			6.450±.122	1.396±.086	21.64±1.39
Minus (—)	3	3	4	27	12	10	1				6.267±.112	1.289±.079	20.57±1.32
Third period													
Plus (+)		3	2	8	26	10	6	4	1		7.283±.125	1.439±.089	19.76±1.26
Check	1	2	3	9	25	9	6	5			7.183±.129	1.478±.091	20.58±1.32
Minus (—)		3	4	7	26	8	7	4	1		7.233±.131	1.499±.092	20.72±1.33
Fourth period													
Plus (+)		1	2	8	28	9	6	5	1		7.417±.117	1.345±.083	18.13±1.15
Check	2	0	3	6	29	10	7	3			7.217±.119	1.367±.084	18.94±1.21
Minus (—)	1	4	4	5	31	7	5	2	1		6.967±.130	1.494±.092	21.44±1.38
Fifth period													
Plus (+)			1	8	21	14	6	7	3		7.817±.124	1.420±.087	18.16±1.15
Check	1	0	3	3	25	10	4	7	5	2	7.933±.158	1.815±.112	22.87±1.48
Minus (—)		2	3	4	25	10	8	5	3		7.617±.136	1.561±.096	20.49±1.31

TABLE 10

Results of continuous selection in speed of propagation in clone 78

Selection	Frequency of class values									Mean	σ	C.V.
	3	4	5	6	7	8	9	10	11			
First period												
Plus (+)		1	7	21	22	7	2			6.550±.088	1.007±.062	15.37±0.97
Check		1	10	21	25	3	0			6.317±.075	0.866±.053	13.71±0.86
Minus (—)		2	10	16	27	3	2			6.417±.090	1.038±.064	16.17±1.02
Second period												
Plus (+)		1	7	11	25	11	4	1		6.900±.103	1.179±.073	17.09±1.08
Check	1	3	5	6	29	9	5	2		6.933±.121	1.389±.086	20.03±1.28
Minus (—)		2	6	10	30	7	4	1		6.833±.102	1.171±.072	17.13±1.08
Third period												
Plus (+)			5	17	31	5	2			6.700±.075	0.862±.053	12.86±0.80
Check	1	2	6	14	27	8	2			6.600±.100	1.143±.070	17.32±1.10
Minus (—)		5	7	13	24	8	3			6.533±.108	1.245±.077	19.06±1.21
Fourth period												
Plus (+)		1	5	10	29	11	3	1		6.950±.095	1.087±.067	15.64±0.99
Check		1	3	7	31	12	4	2		7.167±.102	1.171±.072	16.33±1.03
Minus (—)		2	4	10	27	12	4	1		6.983±.101	1.162±.072	16.64±1.05
Fifth period												
Plus (+)		2	5	9	26	13	3	2		7.000±.107	1.225±.075	17.50±1.11
Check	1	1	5	8	28	12	4	1		6.967±.107	1.224±.075	17.57±1.11
Minus (—)		1	8	8	30	10	2	1		6.833±.097	1.113±.069	16.29±1.03

TABLE 11

Results of continuous selection in speed of propagation in clone 81.

Selection	Frequency of class values									Mean	σ	C.V.
	3	4	5	6	7	8	9	10	11			
First period												
Plus (+)	5	7	10	13	22	2	1			5.833±.123	1.416±.087	24.27±1.58
Check	6	8	10	13	20	2	1			5.717±.127	1.462±.090	25.57±1.67
Minus (—)	8	10	12	23	5	2				5.217±.113	1.292±.080	24.76±1.61
Second period												
Plus (+)	2	3	5	12	30	4	3	1		6.567±.115	1.321±.081	20.12±1.29
Check		1	5	6	33	8	3	3	1	7.133±.111	1.271±.078	17.82±1.13
Minus (—)	1	3	4	7	34	6	3	2		6.833±.113	1.293±.080	18.92±1.20
Third period												
Plus (+)		2	1	10	25	13	5	4		7.283±.110	1.266±.078	17.38±1.10
Check		1	2	11	28	10	6	1	1	7.183±.104	1.190±.073	16.57±1.05
Minus (—)		1	4	7	31	9	5	2	1	7.183±.108	1.245±.077	17.33±1.10
Fourth period												
Plus (+)			1	4	17	26	6	5	1	6.850±.098	1.123±.069	16.39±1.03
Check			1	5	20	24	5	4	1	6.717±.097	1.112±.068	16.55±1.05
Minus (—)			1	2	12	28	10	5	2	7.117±.098	1.127±.069	15.83±1.00
Fifth period												
Plus (+)			1	4	6	32	9	5	2	1	7.200±.108	1.236±.076	17.16±1.09
Check			1	3	10	29	8	6	2	1	7.183±.110	1.258±.077	17.51±1.11
Minus (—)			1	5	5	30	8	7	3	1	7.283±.117	1.343±.083	18.44±1.17

TABLE 12

Differences between the means of the check and those of the selections.

Period	Difference between means of check and plus selections	Difference between means of check and minus selections
	Clone 38	
First	—0.083 ± .124	0.050 ± .125
Second	—0.067 ± .122	0.250 ± .133
Third	0.084 ± .134	—0.167 ± .134
Fourth	0.233 ± .151	—0.366 ± .157
Fifth	0.100 ± .140	0.050 ± .139
	Clone 39	
First	—0.200 ± .167	0.250 ± .168
Second	—0.200 ± .160	0.183 ± .166
Third	0.100 ± .179	—0.050 ± .184
Fourth	0.200 ± .167	0.250 ± .176
Fifth	—0.116 ± .201	0.316 ± .209
	Clone 78	
First	0.233 ± .115	—0.100 ± .117
Second	—0.033 ± .159	0.100 ± .158
Third	0.100 ± .125	0.067 ± .147
Fourth	—0.217 ± .139	0.184 ± .144
Fifth	0.033 ± .151	0.134 ± .144
	Clone 81	
First	0.116 ± .177	0.500 ± .170
Second	—0.566 ± .160	0.300 ± .158
Third	0.100 ± .151	0.000 ± .150
Fourth	0.133 ± .138	—0.400 ± .138
Fifth	0.017 ± .154	—0.100 ± .161

From the data in table 12 it may be concluded that there was no progress obtained in either the plus or minus selection for speed of budding.

Clonal variation in size of frond

As a preliminary selection study the variation in size of fronds in four clones was studied. Selection was later performed in these same four lines. The plants were grown in nutrient solution contained in

TABLE 13

Variation in size in square millimeters in clonal lines of Lemna minor.

Clone	Class values and frequencies																					Mean	σ	C. V.
	1.76-2.25	2.26-2.75	2.76-3.25	3.26-3.75	3.76-4.25	4.26-4.75	4.76-5.25	5.26-5.75	5.76-6.25	6.26-6.75	6.76-7.25	7.26-7.75	7.76-8.25	8.26-8.75	8.76-9.25	9.26-9.75	9.76-10.25	10.26-10.75	10.76-11.25	11.26-11.75	11.76-12.25			
38				1	3	8	6	17	11	11	10	10	7	9	5	1	0	1				6.555 ± .098	1.460 ± .070	20.75 ± 1.03
76				1	4	6	9	8	20	10	6	8	6	6	6	6	3	1				6.735 ± .112	1.665 ± .079	24.72 ± 1.25
79			1	2	2	9	11	18	11	11	15	10	4	1	1	1	1	1	0	0	1	6.220 ± .099	1.472 ± .070	23.66 ± 1.20
81						4	6	12	5	13	11	17	12	12	2	4	2					7.075 ± .090	1.334 ± .064	18.85 ± 0.93

tumblers. One hundred mature fronds were measured from each clone. The results of this study are shown in table 13. From table 13 it is seen that clones 38 and 76 have about the same range of variation. They also approach each other in mean size, which is 6.555±.098 mm² in clone 38 and 6.735±.112 mm² in clone 76. The standard deviations are 1.460±.070 and 1.665±.079 mm² respectively. Clone 79 has the widest range of variation and the least mean size, 6.220±.099 mm². Its standard deviation is 1.472±.070 mm². Clone 81 has the largest mean size, 7.075±0.090 mm², and the least standard deviation, 1.334±.064 mm². Clones 79 and 81 show a significant difference. The difference in the mean is .855±.134.

TABLE 14

Variation in size of fronds grown in nutrient solution and in tap water.

Clone	Period	Culture media	Frequency of class values in square millimeters (mm²)																			
			1.76-2.25	2.26-2.75	2.76-3.25	3.26-3.75	3.76-4.25	4.26-4.75	4.76-5.25	5.26-5.75	5.76-6.25	6.26-6.75	6.76-7.25	7.26-7.75	7.76-8.25	8.26-8.75	8.76-9.25	9.26-9.75	9.76-10.25	10.26-10.75	10.76-11.25
38	First	Nutrient				1	1	4	2	6	14	12	12	15	10	7	6	4	3	2	1
		Tap water	4	13	10	10	24	14	18	6	1										
	Second	Nutrient					2	7	6	11	24	20	11	11	7	1					
		Tap water		1	5	12	22	25	24	8	3										
	Third	Nutrient					6	1	11	14	21	14	8	10	6	4	4	1			
		Tap water	2	7	14	16	20	23	11	5	1	1									
41	First	Nutrient							2	3	12	14	20	11	9	12	8	4	3	0	2
		Tap water		5	14	8	17	18	14	8	11	2	1								
	Second	Nutrient				2	1	4	14	16	19	13	11	9	4	7					
		Tap water	3	7	8	20	19	23	13	4	2	1									
	Third	Nutrient			1	2	2	1	6	3	14	22	16	9	11	5	5	1	1		
		Tap water	1	4	9	14	18	15	13	5	10	3	6	1	0	1					
79	First	Nutrient					1	2	5	7	14	6	13	11	6	13	9	9	1	2	1
		Tap water	1	3	3	8	19	20	12	17	13	3	1								
	Second	Nutrient					3	7	12	10	16	11	11	6	6	5	5	3	3	2	
		Tap water	13	7	11	15	8	13	19	6	6	0	2								
	Third	Nutrient			1	1	3	3	7	7	12	13	11	12	8	11	5	3	0	2	1
		Tap water	2	9	13	20	27	19	7	1	1	1									
81	First	Nutrient			1	0	2	5	5	8	10	14	20	15	8	4	2	4	1	0	1
		Tap water	6	8	19	17	17	23	4	3	2	1									
	Second	Nutrient						2	4	4	6	12	16	19	11	8	3	6	6	2	1
		Tap water	1	5	15	13	20	19	13	9	4	1									
	Third	Nutrient				2	4	2	8	14	8	11	6	11	10	8	8	2	4	1	1
		Tap water	4	9	15	17	14	24	8	2	6	1									

Effect of culture media on clonal size variation

In the study of inheritance of acquired size in *Lemna minor,* which is reported later on in this paper, parallel cultures were grown from each clone in nutrient solution and in tap water. The materials obtained from this experiment may also be examined for the effect of different culture media on variation in size. From each of the four clones used, 38, 41, 79 and 81, three series were grown in different periods of time. One hundred mature fronds were measured from each culture. The results of these measurements are given in tables 14 and 15, the former gives the frequency distributions of the different classes found, and the latter, the different constants calculated.

TABLE 15
Constants from table 14.

Clone	Period	Culture medium	Mean	C.V.	σ	$\sigma_{nut} - \sigma_{tap}$
38	First	Nutrient	7.210 ± .102	20.87 ± 1.04	1.505 ± .072	0.524 ± .086
		Tap water	4.070 ± .066	24.10 ± 1.21	0.981 ± .047	
	Second	Nutrient	6.285 ± .067	15.88 ± 0.77	0.998 ± .047	0.271 ± .058
		Tap water	4.425 ± .049	16.43 ± 0.80	0.727 ± .035	
	Third	Nutrient	6.365 ± .085	19.81 ± 0.98	1.261 ± .060	0.367 ± .074
		Tap water	3.985 ± .060	22.43 ± 1.12	0.894 ± .043	
41	First	Nutrient	7.485 ± .086	17.01 ± 0.83	1.273 ± .061	0.198 ± .079
		Tap water	4.430 ± .073	24.27 ± 0.93	1.075 ± .051	
	Second	Nutrient	6.220 ± .078	18.55 ± 0.91	1.154 ± .054	0.245 ± .069
		Tap water	4.030 ± .061	22.55 ± 1.13	0.909 ± 0.43	
	Third	Nutrient	6.985 ± .088	18.74 ± 0.92	1.309 ± .062	0.022 ± .087
		Tap water	4.575 ± .087	28.13 ± 1.44	1.287 ± .061	
79	First	Nutrient	7.145 ± .103	21.53 ± 1.07	1.538 ± .073	0.533 ± .087
		Tap water	4.710 ± .068	21.34 ± 1.06	1.005 ± .048	
	Second	Nutrient	6.635 ± .108	24.08 ± 1.21	1.598 ± .076	0.332 ± .097
		Tap water	3.980 ± .085	31.81 ± 1.66	1.266 ± .060	
	Third	Nutrient	6.925 ± .106	22.71 ± 1.14	1.573 ± .075	0.753 ± .084
		Tap water	3.825 ± .055	21.44 ± 1.07	0.820 ± .039	
81	First	Nutrient	6.815 ± .093	20.32 ± 1.01	1.385 ± .066	0.438 ± .080
		Tap water	3.755 ± .064	25.22 ± 1.28	0.947 ± .045	
	Second	Nutrient	7.020 ± .095	20.03 ± 0.99	1.406 ± .067	0.450 ± .081
		Tap water	4.160 ± .064	22.98 ± 1.15	0.956 ± .046	
	Third	Nutrient	6.940 ± .114	24.38 ± 1.23	1.692 ± .081	0.674 ± .095
		Tap water	3.930 ± .069	25.90 ± 1.31	1.018 ± .049	

The outstanding result shown by table 15 is that, using standard deviation as the expression of variation, the plants grown in nutrient solution were always more variable in size than those grown in tap water. The differences between the standard deviations of parallel cultures are significant and are, with two exceptions, all well beyond the limits of probable error.

Inheritance of acquired size

The fact has already been pointed out that plants growing under natural conditions have demonstrated their capacity to react readily with favorable medium for growth, not only by a change in shape of the fronds but also by a considerable increase in size, amounting in some cases to more than 100 percent. Likewise it was observed that fronds previously grown in nutrient solution produced offspring which are very much smaller than themselves.

Inheritance of decreased size

An experiment to determine the inheritance of decreased size was made with clones 38 and 41 as follows: From a stock culture of each clone, the same number of buds of the same age were transferred to both tap water and nutrient media and there allowed to propagate until a sufficient harvest of mature fronds could be obtained at any one time. This constitutes the first period of the experiment. In the second period, cultures in both tap water and nutrient solutions were grown from buds from the tap water culture of the first period. At the same time a check culture in nutrient medium was grown. In the third period, tap water and nutrient cultures were similarly grown from the tap water stock of the preceding period and again a check culture was set. There are several months of interval between each two periods to give the plants time to be "acclimatized" in each new medium for growth. From each of

TABLE 16

Mean size in square millimeters of fronds from nutrient solution, grown in tap water, and of their offspring when grown again in nutrient solution.

Clone	Parent mean in nutrient solution	Offspring mean in tap water	Mean when back in nutrient solution	Check in nutrient solution
38	6.555 ± .098	4.070 ± .066	6.315 ± .066	6.775 ± .091
		4.425 ± .049	6.365 ± .085	6.450 ± .092
41	7.120 ± .073	4.430 ± .073	6.220 ± .078	6.615 ± .062
		4.030 ± .061	6.805 ± .090	6.985 ± .088

these cultures one hundred fronds were measured. Table 16 contains a summary of the results of this experiment.

From table 16 it may be concluded that decreased size acquired by nutrient fronds in their sojourn in tap water is not inherited.

Inheritance of increased size

The plan of this experiment is inversely similar to that of the inheritance of decreased size.

This experiment was carried through only two periods. As usual, one hundred mature fronds were studied from each culture. The results of the measurements are shown in table 17.

TABLE 17

Mean sizes in square millimeters of tap-water fronds grown in nutrient solution and of their offspring when grown in tap water.

Clone	Parent mean in tap water	Offspring mean in nutrient solution	Mean when back in tap water	Check in tap water
79	4.750 ± .068	7.405 ± .104	3.980 ± .085	3.825 ± .055
81	3.990 ± .066	6.815 ± .093	4.160 ± .064	3.930 ± .069

From table 17 it is seen that while starved plants grown in nutrient media increase in size by nearly 100 percent, when grown again in tap water reversion to the starved mean may be complete, showing no inheritance of the acquired increased size.

Clonal selection for size of frond

In table 13 the variations in size of clones 38, 76, 79 and 81 have already been shown. Selections for both large and small size were carried out with these four clones as an attempt to shift the means of the lines. Each selection was carried through five periods. Plus selection, or selection for large size, was made by continuously selecting individuals above the mean, and minus selection, or selection for small size, was performed by continuous selection of individuals below the mean. A check culture containing unselected individuals was also grown at the same time with the plus and minus series. The plants were grown in nutrient solution and extreme care was taken to render all controllable conditions as much alike as possible for each set of three cultures. Tables 18 to 21 contain the results of this experiment. The differences between the means of the check cultures and those of the plus and minus selections are placed in table 22 so that the effect of selection may be studied more conveniently.

TABLE 18

Results of continuous selection for large and small size in Clone 38.

Selection	Frequency of class values in square millimeters																					Mean	σ	C.V.
	1.76 to 2.25	2.26 to 2.75	2.76 to 3.25	3.26 to 3.75	3.76 to 4.25	4.26 to 4.75	4.76 to 5.25	5.26 to 5.75	5.76 to 6.25	6.26 to 6.75	6.76 to 7.25	7.26 to 7.75	7.76 to 8.25	8.26 to 8.75	8.76 to 9.25	9.26 to 9.75	9.76 to 10.25	10.26 to 10.75	10.76 to 11.25	11.26 to 11.75	11.76 to 12.25			
First period																								
Plus (+)						6	4	14	18	16	18	13	6	2	2	0	1					6.530±.072	1.073±.051	16.43±0.80
Check				2	4	4	3	10	22	22	11	6	5	4	3	2	1	0	0	1		6.500±.093	1.383±.066	21.28±1.06
Minus (—)					3	5	5	8	13	21	17	15	8	3	2							6.575±.075	1.107±.053	16.83±0.82
Second period																								
Plus (+)			2	2	3	5	9	11	14	13	9	10	9	7	3	1	1	1				6.485±.103	1.525±.073	23.51±1.18
Check				1	1	5	7	9	11	13	19	13	9	5	4	0	1	2				6.775±.091	1.366±.065	20.16±1.00
Minus (—)				1	4	5	10	13	15	9	9	10	13	7	1	1	1	0	1			6.535±.098	1.451±.069	22.20±1.11
Third period																								
Plus (+)						2	4	7	15	20	22	13	9	3	4	0	1					6.830±.069	1.026±.049	15.02±0.73
Check					1	1	6	13	16	22	19	10	4	3	3	1	1					6.615±.072	1.074±.051	16.23±0.79
Minus (—)				2	0	2	5	11	11	24	23	11	8	1	1	1						6.585±.069	1.022±.049	15.52±0.76
Fourth period																								
Plus (+)				1	0	3	2	3	14	18	20	15	11	5	4	3	1					7.025±.078	1.157±.055	16.47±0.81
Check					2	4	1	7	12	14	23	15	7	7	4	3	1					6.965±.082	1.218±.058	17.49±0.86
Minus (—)			1	0	0	2	4	5	15	18	28	18	3	5	0	0	0	0	1			6.755±.069	1.019±.049	15.08±0.73
Fifth period																								
Plus (+)						2	4	1	5	9	19	18	19	7	7	5	4					7.535±.081	1.202±.057	15.95±0.78
Check				1	1	4	2	6	14	12	12	15	10	7	6	4	3	2	1			7.210±.102	1.505±.072	20.87±1.04
Minus (—)			2	1	4	3	3	6	9	11	10	12	8	14	7	6	3	0	0	1		7.175±.115	1.699±.081	23.68±1.19

TABLE 19

Results of continuous selection for large and small size in clone 76.

Selection	Frequency of class values in square millimeters																				Mean	σ	C.V.
	1.76 to 2.25	2.26 to 2.75	2.76 to 3.25	3.26 to 3.75	3.76 to 4.25	4.26 to 4.75	4.76 to 5.25	5.26 to 5.75	5.76 to 6.25	6.26 to 6.75	6.76 to 7.25	7.26 to 7.75	7.76 to 8.25	8.26 to 8.75	8.76 to 9.25	9.26 to 9.75	9.76 to 10.25	10.26 to 10.75	10.76 to 11.25	11.26 to 11.75	11.76 to 12.25			
First period																							
Plus (+)						2	3	5	5	9	13	11	13	16	9	6	5	2	1			7.740±.095	1.413±.067	18.25±0.90
Check							4	5	5	12	13	15	16	9	8	6	5	1	1			7.645±.090	1.331±.063	17.41±0.85
Minus (—)				1	1	3	4	7	10	15	11	11	11	7	6	6	4	2	1			7.275±.106	1.568±.075	21.55±1.07
Second period																							
Plus (+)								2	3	7	9	8	19	15	10	8	7	3	3	3	3	8.485±.100	1.478±.070	17.42±0.85
Check						2	2	3	5	8	9	12	14	15	7	9	4	5	4	1		8.070±.104	1.541±.073	19.09±0.94
Minus (—)						1	1	3	8	9	10	11	12	20	10	6	4	2	2	1		7.930±.093	1.385±.066	17.46±0.86
Third period																							
Plus (+)						1	4	4	5	12	18	14	11	11	7	5	3	2	2	1		7.625±.096	1.425±.068	18.69±0.92
Check						1	2	3	10	17	19	25	7	6	4	3	2	1				7.245±.074	1.101±.053	15.20±0.74
Minus (—)					1	1	3	7	8	17	13	17	7	9	7	5	4	1				7.330±.091	1.355±.065	18.48±0.91
Fourth period																							
Plus (+)				1	2	3	7	11	16	16	12	9	9	5	5	2	1	1				6.715±.093	1.377±.066	20.51±1.02
Check					4	2	8	13	18	14	8	9	4	4	8	6	2					6.745±.102	1.509±.072	22.37±1.12
Minus (—)				1	4	6	13	11	14	11	11	9	8	4	1	6	1					6.465±.100	1.483±.071	22.94±1.15
Fifth period																							
Plus (+)				1	1	1	1	7	10	11	13	13	14	8	7	5	3	2	1	1	1	7.520±.105	1.552±.074	20.64±1.02
Check					1	1	4	5	5	7	18	19	12	13	9	4	1	1				7.465±.084	1.250±.060	16.70±0.82
Minus (—)						1	3	8	10	10	17	16	9	8	6	5	3	2	1	1		7.430±.097	1.431±.068	19.26±0.97

TABLE 20

Results of continuous selection for large and small size in clone 79.

Selection	Frequency of class values in square millimeters																					Mean	σ	C.V.
	1.76 to 2.25	2.26 to 2.75	2.76 to 3.25	3.26 to 3.75	3.76 to 4.25	4.26 to 4.75	4.76 to 5.25	5.26 to 5.75	5.76 to 6.25	6.26 to 6.75	6.76 to 7.25	7.26 to 7.75	7.76 to 8.25	8.26 to 8.75	8.76 to 9.25	9.26 to 9.75	9.76 to 10.25	10.26 to 10.75	10.76 to 11.25	11.26 to 11.75	11.76 to 12.25			
First period																								
Plus (+)				1	2	1	5	15	11	12	16	11	6	7	6	2	3	2				6.940±.099	1.472±.070	21.21±1.05
Check					1	3	7	5	11	12	20	14	13	5	5	2	2					7.000±.085	1.262±.060	18.03±0.89
Minus (—)				1	1	5	6	16	11	8	12	12	11	8	4	2	1	1	1			6.830±.100	1.484±.071	21.73±1.08
Second period																								
Plus (+)					1	3	4	8	11	17	18	8	12	5	4	3	3	1	1	0	1	7.110±.099	1.470±.070	20.67±1.03
Check					1	1	4	5	15	13	20	12	11	9	4	2	1	1	1			7.120±.085	1.263±.060	17.74±0.87
Minus (—)						3	6	11	11	15	19	9	9	7	6	1	1	1	0	1		6.955±.091	1.344±.064	19.32±0.95
Third period																								
Plus (+)			1	0	1	2	5	12	10	8	12	13	8	7	11	4	1	2	1	1	1	7.270±.113	1.674±.080	23.01±1.15
Check					1	6	9	5	12	12	16	12	13	9	3	0	2					6.835±.088	1.312±.063	19.19±0.95
Minus (—)				1	2	4	8	7	16	12	10	13	7	7	6	3	1	1	1	0	1	6.915±.107	1.588±.076	22.96±1.15
Fourth period																								
Plus (+)					2	1	5	6	16	11	11	11	7	5	9	3	4	6	1	1	1	7.435±.118	1.745±.083	23.47±1.18
Check					1	1	6	11	13	13	13	12	9	4	6	4	2	3	2			7.145±.103	1.533±.073	21.45±1.07
Minus (—)				4	2	4	1	8	15	8	13	11	7	10	10	4	1	1	1			7.055±.110	1.629±.078	23.09±1.16
Fifth period																								
Plus (+)				1	1	2	3	6	9	6	12	7	4	7	7	12	6	13	3	1		8.040±.130	1.920±.092	23.88±1.20
Check					1	2	5	7	14	6	13	11	6	13	9	9	1	2	1			7.405±.104	1.538±.073	20.77±1.03
Minus (—)				1	1	2	3	10	15	14	12	10	6	6	5	5	4	4	1	1		7.230±.112	1.662±.079	22.99±1.15

TABLE 21

Results of continuous selection for large and small size in clone 81.

Selection	Frequency of class values in square millimeters																					Mean	σ	C.V.
	1.76 to 2.25	2.26 to 2.75	2.76 to 3.25	3.26 to 3.75	3.76 to 4.25	4.26 to 4.75	4.76 to 5.25	5.26 to 5.75	5.76 to 6.25	6.26 to 6.75	6.76 to 7.25	7.26 to 7.75	7.76 to 8.25	8.26 to 8.75	8.76 to 9.25	9.26 to 9.75	9.76 to 10.25	10.26 to 10.75	10.76 to 11.25	11.26 to 11.75	11.76 to 12.25			
First period																								
Plus (+)					4	1	3	7	10	13	17	11	8	8	9	6	1	1	1			7.220±.100	1.477±.070	20.46±1.02
Check			1	0	2	5	5	8	10	14	20	15	8	4	2	4	1	0	1			6.815±.093	1.385±.066	20.32±1.01
Minus (—)					2	1	7	8	11	8	25	13	8	8	5	3	0	1				6.985±.086	1.279±.061	18.31±0.90
Second period																								
Plus (+)			1	2	4	6	3	9	13	12	18	16	9	3	2	1	1					6.550±.092	1.358±.065	20.73±1.03
Check				2	2	5	5	12	16	15	19	9	5	6	4							6.500±.083	1.232±.059	18.95±0.93
Minus (—)			1	1	2	8	13	9	16	10	14	14	4	3	3	2						6.330±.091	1.342±.064	21.20±1.05
Third period																								
Plus (+)					2	8	6	6	15	11	24	12	10	1	3	0	1	1				6.630±.085	1.256±.060	18.94±0.93
Check			1	0	3	3	7	13	25	15	15	6	4	2	1	2	2	1				6.405±.088	1.298±.062	20.26±1.00
Minus (—)					2	3	6	9	14	17	16	19	7	5	2							6.670±.074	1.091±.052	16.36±0.80
Fourth period																								
Plus (+)					1	2	5	6	11	15	13	11	11	5	4	3	7	4	2			7.375±.109	1.612±.077	21.86±1.09
Check						1	3	4	13	15	15	15	10	7	7	5	2	2	1			7.370±.090	1.334±.064	18.10±0.89
Minus (—)					1	1	3	9	11	11	19	13	8	7	6	5	4	1	1			7.280±.095	1.415±.067	19.44±0.96
Fifth period																								
Plus (+)					1	3	4	8	18	13	11	11	11	8	5	4	2	1				7.035±.093	1.378±.066	19.59±0.97
Check				1	1	1	8	9	13	15	15	14	5	5	4	4	2	1	1	1		6.970±.101	1.495±.071	21.45±1.07
Minus (—)				1	2	6	8	12	18	22	14	6	5	2	1	2	1					6.340±.081	1.194±.057	18.83±0.93

TABLE 22

Differences in square millimeters between the means of the checks and those of the selections.

Period	Difference between means of check and plus selection	Difference between means of check and minus selection
	Clone 38	
First	0.030 ± .117	—0.075 ± .119
Second	—0.290 ± .137	0.240 ± .134
Third	0.215 ± .100	0.030 ± .100
Fourth	0.060 ± .113	0.210 ± .107
Fifth	0.325 ± .130	0.035 ± .154
	Clone 76	
First	0.095 ± .131	0.370 ± .139
Second	0.415 ± .144	0.140 ± .139
Third	0.380 ± .121	—0.085 ± .117
Fourth	—0.030 ± .138	0.280 ± .143
Fifth	0.055 ± .135	0.035 ± .128
	Clone 79	
First	—0.060 ± .130	0.170 ± .131
Second	—0.010 ± .130	0.165 ± .124
Third	0.435 ± .143	—0.080 ± .138
Fourth	0.290 ± .157	0.090 ± .151
Fifth	0.635 ± .166	0.175 ± .153
	Clone 81	
First	0.405 ± .136	—0.170 ± .127
Second	0.050 ± .124	0.170 ± .123
Third	0.225 ± .123	—0.265 ± .115
Fourth	0.005 ± .156	0.090 ± .147
Fifth	0.065 ± .137	0.630 ± .130

If the data in table 22 are examined, and if a significant difference between a selection mean and the mean of a corresponding check is assumed to be at least three times the probable error, it will be found in clone 38 that while the plus means were generally greater and the minus means generally smaller than the means of the checks, no single significant difference was obtained. In clone 76 there seems to be only one important difference, that for the plus selection in the third period. It is hard to account for this seeming effect of selection since in the last two periods of the selection, the difference was not maintained. Moreover, it will be observed that in the same (third) period, the minus series had a greater mean than the check, indicating that some factor, probably cultural, had affected the growth of the plants in the check culture, thus

rendering the opposite differences both unusual. In clone 79 there was entirely no effect of selection in the minus series. In the plus experiments, significant differences were obtained in the third and fifth periods, which may be considered as showing that selection was slightly effective. In clone 81 selection was of no avail. The single important difference obtained in the last period of the minus selection was probably due to the fact that some sort of fungous disease attacked the plants of the minus culture and the effect of the disease on size was not entirely overcome in sampling.

On the whole, it may be concluded that the results of this experiment, to shift the mean size of a clone, showed a very doubtful effect of selection without excluding the possibility that such an effect may be possible.

DISCUSSION AND CONCLUSIONS

Lemna minor is a convenient material for clonal study. It can be grown in artificial media in the laboratory, propagates fairly rapidly and, owing to its small size, it lends itself readily to extensive but well controlled observation and measurements without requiring much laboratory space. In many respects, it is comparable to Paramecium. One advantage it has over the latter is that one can always be sure with it that he is harvesting or sampling for measurement fully matured individuals which have therefore attained their mature size. In Paramecium, there is no way of determining that all the individuals being studied are absolutely fully grown. This fact subjects Paramecium measurement to a grave error, for in comparing the mean size of a group with that of another, the mean size is influenced by the number of immature animals, and it may readily be seen that if one of the groups propagates faster than the other, the former will have at any one time more young individuals than the latter.

While this plant can be grown in tap water alone and in tap water containing soil, the most satisfactory culture medium found, which can be controlled, is a modified PFEFFER's solution. The gradual dwindling of the plants when grown in tap water, especially when no frequent change of this is made, may be due to real lack of mineral food or to the absence of some organic growth-promoting substance which is now called an auximone. BOTTOMLEY (1917) in a recent paper found the presence of this substance essential to the normal and long-time growth of *Lemna minor* in DETMER's standard mineral solution. By placing

water extract of bacterized peat in such a solution, he was able to get continuous, luxuriant growth. Contrary to BOTTOMLEY'S conclusion that *Lemna minor* cannot maintain normal growth in a mineral solution without auximones, it has been grown in this experiment in a mineral solution without the addition of any other substance. BOTTOMLEY'S conclusion is unfair since he did not show that he used the other known standard mineral solutions, any one of which, as the present experiment has proved, may suit the normal growth of the plant.

The characters used as variants in this work are size and shape of frond, and the speed of budding. The length of root is a very unsatisfactory if not a useless character for this purpose on account of its characteristic and probably hereditary twisting habit.

While different strains in shape and size of frond and speed of propagation have been found to exist in a population, the number of these strains is not as large as might at first be imagined. The area of the natural habitat from which the material is collected is undoubtedly an important factor in the obtaining of a larger number of elementary strains, if such larger number exists. The smaller this area is, the more chance there is of finding the population in a high state of freedom from mechanical mixture since, owing to the rapid propagation of the plant, and under the influence of natural selection, a clone may be easily established at any one favorable spot.

Results of clonal selection to shift the mean in speed of propagation or to change the dominant shape of a clone have confirmed the pure line theory. The results of size selection, on the other hand, have not been in entire accord with JOHANNSEN'S idea.

Unusual variations in shape have been observed, but they were not inherited, showing that they were merely somatic or physiological variations.

Lemna minor has been found to respond readily to different culture media. By growing it in an artificial or mineral solution, its natural size has been increased more than 100 percent and the predominating shape of a clone changed, as well as the speed of asexual reproduction hastened. Under such favorable conditions for growth, there was found greater variability in shape and size than under less favorable conditions. Moreover, acquired size, as a result of a change in growing medium, appeared to be non-heritable.

SUMMARY

1. There have been found different races in a population of *Lemna minor* as regards size and shape of frond and speed of propagation.

2. No single case of mutation has been observed in this experiment which covers a period of one and one-half years and which involved several thousands of individuals.

3. In a clone, there was found greater variability among plants grown in a mineral solution than among those grown in tap water.

4. Decreased or increased size acquired by plants through a change of cultural environment during less than a year's time was not inherited.

5. The results obtained in clonal selection either in shape of frond or in speed of propagation are in accord with the pure line theory. Selection to shift the mean size has shown slight effect in one out of ten cases.

The writer is deeply indebted to Professor C. H. MYERS not only for suggesting the subject of this investigation but also for liberal help in securing some of the apparatus and material used, as well as for valuable suggestions and criticisms throughout the experiment and the preparation for this paper. Thanks are also due to Professor H. H. LOVE for aid in some of the biometrical work and to Professor O. F. CURTIS for calling the author's attention to BOTTOMLEY'S work.

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STUDIES ON INHERITANCE IN PIGEONS. III. DESCRIPTION AND LINKAGE RELATIONS OF TWO SEX-LINKED CHARACTERS[1]

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INTRODUCTORY

Some years ago one of us (COLE 1912) reported that the factor producing intensity of pigmentation in pigeons is inherited in characteristic sex-linked fashion, the female being the heterogametic or heterozygous sex. At that time only a few typical examples were presented and a more complete report was promised. Since then our breeding experiments have been continued, and we are now able to present numbers which are really very considerable for such a relatively slow-breeding animal as the pigeon. In this paper we include a fuller report on this factor and a first discussion of another sex-linked color factor of peculiar interest and also give what data we have on the mutual linkage of these two. The second factor, which frequently produces a condition termed for

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convenience "dominant red," was mentioned in an earlier report (COLE 1914, p. 347). Its identification explains why investigators have presented conflicting evidence on the inheritance of red in pigeons.

Our breeding experiments with pigeons have been continuous since 1907, having been begun in that year at the RHODE ISLAND AGRICULTURAL EXPERIMENT STATION and continued there until 1910, when they were transferred to Wisconsin. The present tabulations include all pertinent data for the entire period. The junior author became associated in the work in 1911.

DESCRIPTION OF THE CHARACTERS

1. *Intensity*

The differences between the intense and the dilute series of colors in pigeons have been quite fully discussed in one of the reports mentioned (COLE 1914), and consequently need not be considered in detail at this time. The intensity factor, which we have designated *I*, acts as in mammals, with equal effectiveness on both black and red pigment. LLOYD-JONES (1915, p. 481) has shown that in the pigeon the effect of *I* is to increase the amount of pigment in the feathers, whether black or red, to approximately three times what it would be if *I* were absent. The factor affects the pigment in all parts of the plumage.[2]

As a consequence of the above we have in domestic pigeons the intense series of colors, black, blue and red, corresponding respectively to dun, silver and yellow of the dilute series. In most cases there is no difficulty in differentiating the two conditions, even though there are only one or two pigmented feathers in the plumage. It sometimes happens, however, that a black bird will show a lower grade of pigmentation in the juvenal plumage than after the first moult. In a few cases there may be some uncertainty as to whether a bird dying young is black or dun. In other cases the pigment in certain parts of the plumage may be altered by modifying factors so that its condition with respect to intensity is likewise uncertain, and these cases may be further complicated if the birds are nearly all white with the pigment confined to such modified regions. Practically no cases arise, however, in which the condition cannot be definitely determined by careful examination and comparison. Doubt-

[2] No noticeable difference has been observed in the color of the eye in the living bird, the pupil appearing jet black in those that are dilute as well as in those that are intense. In this respect the case is similar to that of mammals (see footnote, p. 186). It is possible nevertheless, that microscopic examination might reveal differences in the amount of pigment in the retina.

ful cases were checked, whenever possible, by subsequent breeding tests, and the breeding test is, of course, the only method of determining the presence or absence of *I* in birds whose plumage is entirely white.

There appears to be a rather definite correlation between amount of down on the newly hatched squab and the intensity of the definitive plumage. A complete study of this has not yet been made, but our records reveal 64 cases in which the down condition was noted and the plumage color is known. Among these there are only four positive exceptions to the rule that intense birds had "abundant" down when hatched, while on the dilute birds it was "sparse." Whether the recorded exceptions are real or due to lack of uniformity in description we are not at the present time prepared to state, for while the differences in amount of down are usually quite distinct, in other instances they appear to intergrade.

This marked correlation of sparse down with dilute pigmentation was brought to DARWIN's attention by TEGETMEIER and is mentioned by him in "Animals and plants under domestication" (1868) and elsewhere. He says:

"Mr. TEGETMEIER has informed me of a curious and inexplicable case of correlation, namely, that young pigeons of all breeds which when mature become white, yellow, silver (i.e., extremely pale blue), or dun-coloured, are born almost naked; whereas pigeons of other colours are born well-clothed with down."[3]

It is a matter of much interest that STRONG (1912 b) has noted a similar relation of intensity of pigmentation to abundance of natal down in the Ring-dove (*Streptopelia risoria*), the blond variety having much down while the whites have little. STRONG makes this as a general statement and does not say whether exceptions ever occur. We also have noted the same difference in Ring-doves. The matter of exceptions is of importance, since if the two conditions are really completely correlated it would indicate either two factors completely linked or else two very different physiological effects of a single factor. If true exceptions occur, it would appear that abundance of down depends upon a separate sex-linked factor, closely, but not completely linked to *I*. The fact that a

[3] Reference to this observation occurs in the unpublished DARWIN-TEGETMEIER correspondence deposited in the library of the New York Botanical Gardens. It seems probable that the whites referred to were genetically dilutes; for if the correlation is between the down condition and the factor for intensity of pigmentation rather than the condition of expression of that factor, whites carrying *I* would be expected to have abundant down in the nestling stage. Our records do not permit a decision at present on this point.

parallel condition occurs in the Ring-dove suggests that the difference between the blond and white types is really an intensity difference and that the sex-linked factor which is responsible is homologous to the factor *I* in the domestic pigeon.

2. *Dominant red and gray*

That the uniform red color found, for example in the Tumbler breed of pigeons, is a simple Mendelian recessive to black was first reported by the senior author (COLE 1909) and later (COLE 1914) elaborated in more detail. The differentiating factor was given the symbol *B*. In the presence of *B*, the plumage color is black, blue, dun or silver; in the absence of *B* a uniform red or yellow.[4]

At the time the 1914 paper was being written, it had become evident that a second factor is capable of producing reddish pigmentation in pigeons, this fact being referred to on pages 326, 335 and 347 of that paper. This factor alters the expression of *B* so that at least some birds carrying both *B* and *A* are a distinct but not uniform red. Such red birds mated to homozygous blacks have red offspring, and therefore red of this type may conveniently be referred to as "dominant red" to distinguish it from the recessive red (type *ba*) mentioned above. The *A* factor, like *I*, is sex-linked in inheritance, the female never being homozygous for dominant red. The recessive red is of course inherited independently of sex.

As we at present interpret our breeding evidence, the primary characteristic of the *A* factor is its modification of black and not the production of red pigment, for we have secured a series of sex-linked gray colors, dominant to black, produced by what is apparently this same *A* factor. Whether an *A* bird shows red or gray probably depends on some unidentified factor or factors which modify the action of *A*; although the possibility that the gray-producing *A* is a third allelomorph in the set must be considered. If either of these interpretations proves to be true, no confusion will result from combining the reds and grays in discussing the *A* factor and its linkages, and this we have done in our tables. If further work should show that neither is tenable, and that the two conditions are produced by separate sex-linked factors, tables 2 and 4

[4] To make the interpretation analogous to that now current in mammals, *B* may, if desired, be considered an extension factor for black pigmentation. In the absence of *B*, black is confined to the retina. This view may prove useful in the explanation of certain recessive red birds which bear a few dark-colored (black?) feathers, this unusual condition being interpreted as partial extension of black.

of this paper would lose some of their value and table 3 become meaningless. However, our limited evidence indicates that the two general conditions are not produced by separate factors, a point which we hope to discuss on another occasion.

Red and yellow birds carrying *A* but lacking *B* (formulas *bAI* and *bAi*) are indistinguishable in appearance from ordinary recessive reds and yellows (*baI* and *bai*). We have not found such birds among any of the recognized breeds that we have experimented with but have formed them without difficulty in recombinations. Pigeons of the dominant red type of coloration (*B* present) commonly show an admixture with blue of a peculiar shade usually quite distinct from the blue (which is really a light gray) seen in the familiar blue with black wing bars that occurs in various domestic varieties and in most specimens of the wild Rock pigeon. In the dominant red the relative amount of red and blue is subject to much variation, individuals occurring in which the red is very greatly reduced. The individual feathers are seldom a uniform red but show bluish towards the base where the red color runs out. The red is somewhat deeper than the color of recessive reds. The difference between the blue on these birds and that of the ordinary blue (*a*, with black bars) appears to be due to varying amounts of reddish pigment which occurs in most of the blue feathers, giving a somewhat "warmer" tone than that of the ordinary blue. In very clear red-barred birds this difference is not very considerable if attention is confined strictly to the clearest areas, such as the lesser wing coverts. In birds that are more red the blue takes on a distinctly brownish tinge.

A characteristic of all birds bearing both *A* and *B* is a peculiar lightening of the color of the tail that we have often in our records referred to as a "washed-out appearance." The tail is in all cases noticeably different from the body, even in birds which without *A* would have their pigmented areas uniformly colored. This peculiarity is frequently useful in classifying white splashes as it is the most persistent characteristic of *A* birds.

Some of the red and apparently all of the gray *AB* birds show black flecks or patches or both scattered over the body. This condition has, however, not yet been analyzed. We have seen nothing corresponding to it in non-*A* individuals.

Among pigeons bearing the *B* factor but not *A*, there are three general types, the uniform black, the check and the blue-barred. These have been described in some detail by COLE (1914). Each exhibits certain

variations. The uniform black may be glossy or dull. The checks show differences in the relative proportions of black and the lighter contrasting colors, while the latter in turn may be blue or a dull somewhat grayish black generally called "smoky." The barred types have two black wing bars and a black terminal tail band; the rest of the wing and the contour feathers may be a clear gray blue or a dark smoky blue. These categories are perhaps not as sharp as might be inferred from the above statements, as there are certain intergrades which may, however, for our purposes be ignored.

Both the dominant red and the gray series have types corresponding more or less closely to those of the black series. Birds of the red series that would be uniform black in the absence of *A* have wing coverts, head, neck and back of a fairly uniform red. They are light in the outer primaries, rump, tail, flanks and lower belly. These light areas are sometimes a rusty blue, sometimes a palish brown. Red checkers are readily recognized. The contrasting color on the wing coverts is either a rusty blue or a lighter brownish red, which we think correspond respectively to the blue and smoky contrasting colors in black checks. The red checks do not show a terminal tail band, the tail being approximately as in the "uniform" dominant reds. The type in the dominant red series corresponding to the blue-barred of the black series is called silver[5] by Homer fanciers, at least in this country, but we shall call it red-barred. It has two distinct red wing bars but the general body color is bluish. This blue is sometimes very pale, sometimes dark and mixed with red. These differences doubtless correspond to the gradation from clear blue-gray to a dark smoky color found in barred birds of the black series. The tail is generally light bluish or brownish blue and faded in appearance. There is usually a deposition of brownish pigment on the under side of the tail feathers in a poorly defined area just anterior to the region that the terminal band occupies in blue-barred birds. This pigmentation may usually be seen from the dorsal surface. In the red-barred birds, then, the tail-band region commonly shows up lighter than

[5] The term "silver" is used in other publications of this series of studies for the dilute blue, a type of coloration in which the wing bars and tail band are dun, and this is the usage of most fanciers. Those who call the birds with red bars "silvers" sometimes refer to the birds with dun bars as "silver-duns." In this paper we shall continue to use the term silver in the same sense as heretofore so as to avoid misunderstanding, since to use silver in the sense of the Homer fanciers would introduce very great inconsistencies into our terminology. "Red-barred" is the best descriptive term that has occurred to us. Adopting this however we should, to be consistent, call blue birds "black-barred" and silvers "dun-barred," but the change hardly seems necessary.

the rest of the tail. The statement made by COLE (1914, p. 326) that the terminal band is red was inexact and should be corrected.

A similar series of types presumably exists among the grays though these have not been as yet thoroughly worked out. We however have not yet secured gray checks. The most nearly uniform grays are very nearly the same shade in all parts of the plumage, even the primaries and tail being but very little lighter. As already mentioned we have not yet secured any of this type which were entirely free from black flecks, and although the red pigment may be reduced to little more than a trace in the position of the wing bars, this also appears never to be entirely absent. When more red occurs in the wing bars, red pigment appears also on other parts, particularly the head and neck, resulting in a red-barred gray corresponding to the red-barred blues.[6]

Mention has been made in the foregoing discussion, of the effects of the *A* factor only on the *intense* series of colors, red, black and blue. In other words, the presence of the intensity factor, *I*, has been assumed in all cases. What has been said of the effects of *A* applies, however, in the same way to the dilute condition of these colors. A *BA* bird lacking *I* is yellow, just as an ordinary red bird (*ab*) is yellow if *I* is absent, and the dilute condition of the bird with red wing bars (*BsAI*) is similarly a modified bluish or grayish, with yellow instead of red wing bars.

The *A* factor is undoubtedly of wide distribution in different breeds of pigeons. By breeding tests we have identified it in Homers, in white clean-legged Tumblers, in white-muffed Tumblers, in white Fantails and in red-and-white Tumblers showing the Baldhead pattern, all of which were secured from widely different sources. It was apparently present in many of the birds which BONHOTE and SMALLEY (1911) used in their experiments. The birds referred to by them as "dark mealy," "light mealy," and "white mealy," all of which are figured on plate XXV accompanying their paper, obviously carry the *A* factor, the first-mentioned either being identical with, or at least resembling very closely, what we have termed red-barred. On the other hand, their "blue," "silver" and "chequer," figured on plate XXIII, correspond to the types for which we use the same terms, and as obviously do not carry *A*. Furthermore the birds they designate as "grizzles" (*loc. cit.*, plate XXIV) are apparently modified *a* types, a fact which is rendered more conclusive by a comparison of the grizzle and mealy feathers depicted on plate XXVI. This interpretation

[6] Descriptions in some detail of specimens selected to represent several of the dominant red and gray types will be found as an Appendix to this paper, p. 202.

accords with their statement that mealy is dominant to grizzle and, in the light of our own investigations on the *A* factor, helps to clear up their difficulty in explaining "the predominance of one sex in certain colours." In a footnote on p. 617 they say:

"This most interesting question has not been dealt with in the present paper, as we have not yet fully investigated the results; but we may mention that a large proportion of the White Grizzles are ♀'s, and in the Light Mealies by far the larger number are ♂'s; we have also bred a certain number of Cream Mealies, and these have all been ♀'s."

If BONHOTE and SMALLEY had tabulated their results by individual matings they doubtless would have found that in certain matings all the males were mealies and all the females grizzles, since this would be the expectation whenever grizzle cock was mated to mealy hen. Their last statement, that all the "cream mealies" bred were females, is difficult of explanation, since by hypothesis, if these birds also carried the *A* factor, they would be expected to be males if there were any restriction as to sex. We are not certain, however, just what the authors mean by "cream" mealies, since they do not appear to be described or mentioned elsewhere in the paper.

Evidence that the *A* factor was involved in STAPLES-BROWNE's experiments on inheritance of color in pigeons is not so definite, although his statements (STAPLES-BROWNE 1908, p. 70) that possibly "two types of reds may eventually be demonstrated," and (STAPLES-BROWNE 1912, p. 133) that the question of the inheritance of red and yellow is a complicated one, may have been induced by complications due to the presence of this factor in some of his birds. Among other breeds he employed Fantails in his experiments, and, as already stated, we have identified the *A* factor in individuals of this breed.

A series of experiments recently reported by NUTTALL (1918) also involves this same factor. Nuttall worked with the "Racing Pigeon," which is probably the same as our Racing Homer. He divides his birds into four color varieties, viz., blue, blue checker, mealy and red checker. He finds red (of the red checker or mealy) dominant. His use of blue is the same as ours, namely blue birds with black wing bars and tail band; blue checkers are blues plus checking. Regarding the mealies and red-checkers he states:

"The colour of the so-called mealy birds is difficult to describe. The ground colour is somewhat like that of fine oat-meal; the wing-bars are reddish—approaching the colour of damp sand. The mealy birds differ in two salient points from the blues—the wing- and tail-quills are, as a rule, pale in colour, and the tail-bar is absent.

"The red-chequered birds stand in the same relation to the mealies as the blue chequers do to the blues, i.e., they are mealies with the addition of chequering. The wing-quills and tail-quills are generally pale in colour, there is no tail bar."

NUTTALL finds the red (of the red checker or mealy) dominant to blue, and check dominant to its absence,[7] and assumes two sets of allelomorphs to account for the four color types. These are: *R*, presence of red; *r*, absence of red; *C*, presence of checking; and *c*, absence of checking. The types may be represented by phenotypic formulae as follows: *RC*, red checker; *Rc*, mealy; *rC*, blue checker, *rc*, blue. On the strength of the descriptions quoted above and the behavior of red in relation to black (of the blues) in the breeding tests, there can exist no doubt whatever that NUTTALL's red checker is the same as our dominant red check, and that his mealy corresponds to the mealies of BONHOTE and SMALLEY (1911) or what we have termed red-barred. NUTTALL's factor *R* is therefore identical to our *A*, but owing to the fact that he lumped his data and did not consider the results of individual matings separately, he apparently gained no inkling of its sex-linked nature.[8]

EXPERIMENTAL RESULTS

1. *Inheritance of the I factor*

In the case of a sex-linked factor, only three types of matings provide evidence of segregation. For the *I* factor, these are:

1. ♀ *I–* × ♂ *Ii*; expectation, 2 intense ♂♂ to 1 intense ♀ to 1 dilute ♀.
2. ♀ *i–* × ♂ *Ii*; expectation, 1 intense ♂ to dilute ♂ to 1 intense ♀ to 1 dilute ♀.
3. ♀ *I–* × ♂ *ii*; expectation, 1 intense ♂ to 1 dilute ♀.

Table 1 gives a summary of the results obtained from all three types of matings. This covers the offspring of 78 matings or families of type 1, 70 families of type 2, and 60 families of type 3. A detailed presentation of the results from individual matings is omitted in the

[7] The dominance of check was first mentioned by BATESON in 1909 on the authority of STAPLES-BROWNE, and later verified by BONHOTE and SMALLEY (1911) and by COLE (1914, p. 335).

[8] NUTTALL summarizes his results according to phenotypes crossed and assumes that the possible genotypes in each phenotype are present in equal proportions—in itself a dangerous assumption with such small numbers. Unfortunately, furthermore, in considering the possible genotypes of red checker he makes an error (considering *RCrc* and *RcrC* as different) which vitiates his calculations throughout. Consequently, while his expected appearances are correct as to classes, they are faulty with respect to numbers.

present report as it would occupy considerable space and would serve little purpose. It is to be understood that all matings meeting the requirements and showing segregation with respect to intensity have been included, whatever the color. In some cases the *A* factor is involved, and in many the birds may have been entirely white except for a few feathers by which the intensity could be determined; or in some instances they were entirely white and the question as to whether they carried *I* was determined from breeding results.

The first thing to be noted from this table is that with one possible exception all records fall into the classes which would be expected from the nature of the respective matings.[9] The doubtful case is a single dilute male recorded in type 1. As a matter of fact the descriptions of this bird show uncertainty, for it was described on September 15, 1908, when somewhat less than a month old, as "apparently a good white bird except for flecks of *red* at the tips of some of the primaries, secondaries and greater primary coverts." After molting, however, it was again described on January 23, 1909, as "pure white except for a very little *yellow* at the tip of one greater primary covert, in secondary of right wing, and in one primary of left wing." This individual was included as dilute in the earlier report on the basis of the later description, but it now seems extremely probable that the first description is the correct one. On account of the uncertainty, however, the bird is not included at all in the present computations. It is clear, considering the contradictory descriptions, that it should not be counted as an exception to the sex-linked segregation of *I*. We thus find for the intensity factor in pigeons no cases of "partial sex-linkage" such as those reported in the Ring-dove

[9] There are two matings, not included in the table, in which dilute birds appeared where they were not expected at all. A white female (364A) with only a few dark-pigmented feathers was bred to a homozygous black male, and among the offspring was a female (687B) which was white except for a few *yellow* feathers. She in turn was bred to a black male homozygous for intensity, and among their offspring also there was a white bird, this time a male (909A), with a few yellow feathers. We have noted these birds as "aberrant yellows" and as yet have no explanation of the case. It is complicated by the fact that the birds were so largely white, and it is known, furthermore, that some of them carried the *A* factor, which complicated matters still further. It should perhaps be mentioned furthermore that these early results were obtained at a time when the matings were not controlled as closely as they have been in the later years of the work. Until 1911 the mated pairs were not isolated but were kept together in large pens, though special care was taken to prevent cross-mating and to detect it if it occurred (COLE 1914, p. 318). Beginning in 1912 the use of separate pens for pedigreed matings was begun, and this practice was extended so that there were very few non-isolated pairs in 1914 and all matings have been strictly controlled since that time.

TABLE 1
Inheritance of I.

Type of mating		♂♂		♀♀		Sex?		Color?		Total, color		Total, sex		Sex ratio
		I	*i*	*I*	*i*	*I*	*i*	♂♂	♀♀	*I*	*i*	♂♂	♀♀	♂♂ : 100 ♀♀
♂ *Ii*	Obtained	220	(1)	90	88	20	5	79	61	330	93	300	239	125
1. ———														
♀ *I-*	*Expected*	*199*	——	*99.5*	*99.5*	*19*	*6*	*70*	*70*	*317*	*106*	*269.5*	*269.5*	*100*
		204	——	*97*	*97*	*19*	*6*	*72*	*68*	*320*	*103*	*276*	*263*	*105*
♂ *Ii*	Obtained	106	73	87	85	11	12	94	91	204	170	273	263	104
2. ———														
♀ *i-*	*Expected*	*87.5*	*87.5*	*87.5*	*87.5*	*11.5*	*11.5*	*92.5*	*92.5*	*186.5*	*186.5*	*268*	*268*	*100*
		90	*90*	*85.5*	*85.5*	*11.5*	*11.5*	*95*	*90*	*192*	*182*	*275*	*261*	*105*
♂ *ii*	Obtained	124	——	——	115	13	14	37	31	137	129	161	146	110
3. ———														
♀ *I-*	*Expected*	*119.5*	——	——	*119.5*	*13.5*	*13.5*	*34*	*34*	*133*	*133*	*153.5*	*153.5*	*100*
		122	——	——	*117*	*13.5*	*13.5*	*35*	*33*	*136*	*130*	*157*	*150*	*105*
Total sexes	Obtained	——	——	——	——	——	——	——	——	——	——	734	648	113
	Expected	——	——	——	——	——	——	——	——	——	——	*691*	*691*	*100*
		——	——	——	——	——	——	——	——	——	——	*708*	*674*	*105*

by STAPLES-BROWNE (1912) and STRONG (1912 b).[10] The attempt was made by BRIDGES (1913) to explain these exceptions on the assumption of a pair of sex chromosomes in the female dove and a discrete "sex-differentiating factor" located near the factor for plumage color. Later, however, he puts forward the opinion that "non-disjunction offers an alternative explanation which seems more plausible" (BRIDGES 1916, p. 157). We are inclined to believe that the records are more probably in error.

Even a casual examination of the table reveals an almost consistent excess of males and of intense birds beyond expectation; only in a few instances is this not true, and then the differences are very slight. An excess of intense birds was noted and commented upon at the time of the earlier report on the segregation of the *I* factor (COLE 1914, p. 360). In that report sex was not considered. It was then suggested that the discrepancy between observed and expected ratios might have been due in part at least to errors in determination of the color in some instances. This explanation has now, however, been definitely ruled out, since it has been found that such errors were at most exceedingly infrequent in the earlier records, and there is even less probability of their occurrence since that time. Nevertheless, although the total number of cases has been more than doubled since reported in 1914, the excess of intense birds is in general even greater in proportion than it was then.

The fact that there is an excess both of males and of intense birds, suggests at once a causal relation between the two, especially as the intensity factor is sex-linked. It seems very probable, in fact, that this is the true explanation of the excess with respect to intensity. It does not, however, explain why the males should be so much in excess in many cases. If the normal ratio of males to females in a heterogeneous population of pigeons be accepted as 105 : 100 (COLE and KIRKPATRICK 1915, p. 465),[11] it will be seen that in the matings of

[10] In table II of STRONG's paper (l.c., p. 301) and on page 313 he states that he obtained three blond females from mating of white male to blond female, from which only blond male and white female offspring would be expected. Tables XXI and XXII, however, which present his results in detail, show only two recorded exceptions (in mating 29) and this is the number he himself states he obtained in another paper (STRONG 1912 a, p. 443). This may perhaps cast some doubt on the validity of the two recorded exceptions.

[11] As stated in a footnote on the page referred to, a count of all sexes determined to December 1, 1914, gave 919 males to 881 females, a ratio of 104.31 : 100. A tabulation of all sexes recorded up to January 1, 1918, shows 1632 males to 1537 females, or a ratio of 106.18 : 100. This again indicates that the average ratio for our population tends to vacillate around 105.

type 2 the results came fairly close to this norm; but in the other two types of matings the ratio was much higher. Particularly is this so for type 1, where the males to females are as 125 : 100. The numbers on which this ratio is based are too large for this to be a chance deviation.

In presenting for comparison the expected results with those actually obtained, the expectations are given based both on normal, unmodified Mendelian segregation of sex (ratio 100 : 100) and of the factor for plumage intensity and also on the assumption that the average sex ratio is for some unknown reason 105 : 100. It will be observed that the latter figures tend to correct in considerable degree the discrepancies which occur in the case of the former. Even so, however, a decided excess still remains to be explained. We are unable at present to suggest any well substantiated explanation, but a careful study of the individual matings, particularly of type 1, where the excess noted is greatest, brings out certain facts which are suggestive. These are not definite enough to warrant the inclusion of the detailed data here, but it is hoped by further breeding in selected families to secure large enough numbers for more adequate analysis. The most significant fact apparent is that the great excess of males, and consequently of intense individuals, appears to be due mostly to widely divergent ratios in certain families and not to a general tendency in all. If we were to assume a sex-linked lethal factor in such families, closely associated with *I*, the excess of male offspring could be accounted for. The ratio of males to females in lines carrying the lethal should be 2 : 1, and in a few matings where there is a fair number of offspring the proportions approximate this ratio. The closer the association of *I* with the lethal factor, the nearer would the ratio of intense to dilute offspring also approach that of 2 : 1. The proportions of the sexes and of intense and dilute birds in any population would accordingly depend upon the relative number of lines carrying the lethal and the closeness of association between the lethal and the factor *I*. While this hypothesis is admittedly hung on a very slender thread of data, it at least has the advantage that it can be tested by definite and known experimental methods.

2. *Inheritance of the A factor*

In table 2 are shown the data on the inheritance of the *A* factor. Here again it will be noted that all fall in the expected categories, with two possible exceptions,[12] a recessive (*a*) male and a dominant (*A*) female,

[12] An exception not included in the table occurred in the case of ♀ 687B, one of

in one of the matings of type 3. The records themselves in this case contain no indications of confusion, but it seems significant that the two birds in question were nestmates, and that if the sexes were simply reversed, they would fall into their proper classification.[13] This happens, furthermore, to be one of the relatively few instances in the earlier part of the work in which the recording of the sex was done by an assistant in the absence of the senior author, and before the junior author was associated with the work. All things considered, the probability of a reversal of the sexes having been made in recording seems strong enough to warrant these birds not being counted as exceptions; instead, they have simply been omitted from the computations altogether.

There is a striking difference in the sex ratios in table 2 as compared with table 1. In table 1 there was an excess of males in all three types of matings, as has been seen. In table 2 just the opposite is the case in the first two types of matings, while in the third there happens to be exact equality. While in most cases the observed numbers are fairly close to expectation on a basis of equality of males and females, they are still farther away from expectation on a basis of 105 : 100. The very low ratio of males in type 1 cannot be given great weight because of the relatively small numbers, but larger numbers are involved in the other matings, and there can be no doubt that the general deficiency of males in table 2 as compared with table 1 is significant. The theory suggested to explain the excess of males in the former instance cannot be used to

the "aberrant yellows" already referred to in the footnote on p. 192. The recorded male parent of this bird was a black (*aa*) and the mother (presumably *A*—) was white with a few dark-pigmented (dominant red?) feathers. In such a mating none of the female offspring should carry *A*, but ♀ 687B evidently did. Two other birds (1533B_2 and 1608V) having the *A* factor are recorded from parents neither of which had it. These birds also are related, the father of 1608V being a brother of 1533B_2, a fact which may have significance. Since, however, these cases are so irregular, and as no explanation is at present apparent, they have been omitted from consideration in the present connection. Cross mating or confusion of records is possible, but we have found nothing except the discordant results to indicate that anything of this kind is responsible. The later cases are being continued for further investigation.

[13] In this connection it might also be mentioned that in the early work at Rhode Island the letters A and B were used to designate the squabs hatched from the first and second eggs respectively, the records being intended for use in a study of the relation of order of laying of the eggs to the sex of the resulting offspring (Cole and Kirkpatrick 1915). When the identity of egg and squab was lost the symbols X and Y were used to indicate that fact. It is possible that this may have some bearing on the apparent reversal of the sex records in the present case, for the nestmates in question were an X and Y pair and the assistant who made the record of sexes, knowing that they would be useless in that investigation, might not have made the entries with his usual care.

TABLE 2
Inheritance of A.

	Type of mating		♂♂		♀♀		Sex?		Color?		Total, color		Total, sex		Sex ratio
			A	*a*	*A*	*a*	*A*	*a*	♂♂	♀♀	*A*	*a*	♂♂	♀♀	♂♂: 100 ♀♀
1.	♂ *Aa*	Obtained	14	—	8	8	6	1	6	13	28	9	20	29	69
	♀ *A-*	*Expected*	*15*	—	*7.5*	*7.5*	*3.5*	*3.5*	*9.5*	*9.5*	*28*	*9*	*24.5*	*24.5*	*100*
			15	—	*7.5*	*7.5*	*4*	*3*	*10*	*9*	*28*	*9*	*25*	*24*	*105*
2.	♂ *Aa*	Obtained	57	48	53	49	4	6	34	41	114	103	139	143	97
	♀ *a-*	*Expected*	*51.75*	*51.75*	*51.75*	*51.75*	*5*	*5*	*37.5*	*37.5*	*108.5*	*108.5*	*141*	*141*	*100*
			53	*53*	*50.5*	*50.5*	*5*	*5*	*38*	*37*	*111*	*106*	*144*	*138*	*105*
3.	♂ *aa*	Obtained	65	(1)	(1)	64	1	4	27	28	67	69	93	93	100
	♀ *A-*	*Expected*	*64.5*	—	—	*64.5*	*2.5*	*2.5*	*27.5*	*27.5*	*68*	*68*	*93*	*93*	*100*
			66	—	—	*63*	*3*	*2*	*28*	*27*	*70*	*66*	*95*	*91*	*105*
	Total sexes	Obtained	—	—	—	—	—	—	—	—	—	—	252	265	95
		Expected	—	—	—	—	—	—	—	—	—	—	*258.5*	*258.5*	*100*
			—	—	—	—	—	—	—	—	—	—	*265*	*252*	*105*

explain the deficiency of them here; but if the suggested lethal were entirely absent the ratio of males should not be higher than 100. If the two sets of data are combined, duplicates being counted only once, thus making up a larger sample of the total population of the lofts, the total numbers are 898 males to 832 females, or a ratio of 108:100. This number is fairly close to 105, which has been taken as the average of the general population. It would doubtless have approximated it even more nearly had the number of birds involving the *A* factor been as large as that included under *I*.

The foregoing illustrates how general population averages are derived from composites and in themselves may have little if any direct biological significance.

3. *Linkage relations of I and A*

Since *I* and *A* are both sex-linked in their inheritance, it is naturally to be expected that they should show some linkage to each other. We have been endeavoring for the past four seasons, by experiments designed definitely for that purpose, to obtain sufficient data to establish such linkage beyond reasonable doubt and to obtain a reliable measure of its intensity. GOODALE (1917) has recently reported crossing over of sex-linked factors in the fowl, where it occurs in the male as is the case in Lepidoptera. This is apparently the first published record for birds. We have been aware of crossing over in the male pigeon ever since the second sex-linked character was recognized in 1913; our difficulty has been to demonstrate that there was any linkage. Only relatively few matings could be devoted to this purpose, and reproduction in pigeons is so slow that our numbers are still inadequate to give anything like an exact measure of crossing over. While, therefore, we do not claim that the figure obtained to date indicates the precise amount of crossing over between these two factors, we do feel nevertheless that the divergence from equality of crossover and non-crossover types among the offspring is sufficient to indicate that an appreciable, though probably relatively slight, degree of linkage exists.

In table 3 the details are given of those matings used in the linkage computations. In the upper part of the table are matings of males who received both dominant factors from one of their parents and both recessives from the other. The combination given first is that received from the father and the second is that from the mother. Thus ♂ 896B in mating 1320 received the recessive factors *ia* from his father and the dominant factors *IA* from his mother; and so for the others. The results

TABLE 3
Matings showing linkage of I and A.

Number	Mating				Offspring			
	♂ parent		♀ parent		Non-crossover		Crossover	
	Number	Formula	Number	Formula	*IA*	*ia*	*Ia*	*iA*
1320	896 B	*ia.IA*	1028 B	*ia*—	0	2	0	1
1329[14]	711 A	*ia.IA*	655 A	*IA*—	0	1	0	0
1450	896 B	*ia.IA*	450 Y	*ia*—	2	0	0	0
1511	1470 B	*ia.IA*	847 A	*ia*—	5	4	3	2
1512	1457 B	*IA.ia*	1332 B	*ia*—	4	0	2	0
1580	1061 H	*IA.ia*	781 B	*ia*—	2	2	0	1
1602	1457 B	*IA.ia*	1304 K	*ia*—	6	1	3	4
1617	1457 F	*IA.ia*	1325 C	*ia*—	1	1	2	1
1620	1061 H	*IA.ia*	1471 E	*ia*—	1	0	0	0
1624	1511 E	*IA.ia*	1470 K	*ia*—	1	1	0	0
Totals					22 *Ia*	12 *iA*	10 *IA*	9 *ia*
1365	968 B	*Ia.iA*	903 A	*ia*—	1	0	2	0
1456[14]	1167 A	*iA.Ia*	1432 A	*Ia*—	2	1	1	1
1523	1335 M	*iA.Ia*	781 B	*ia*—	5	1	3	3
Totals					8	2	6	4

Summary: 44 non-crossovers, 29 crossovers.
Percent crossing over, 39.72.

of these matings tend, therefore, to show "coupling." The three males in the lower part of the table received one dominant and one recessive factor from each parent, and their offspring accordingly indicate a corresponding "repulsion" of *I* and *A*. Of the 73 offspring obtained in these 13 matings, 29 were crossovers and 44 non-crossovers. Thus crossing over occurred in 39.72 percent of the cases. Although with such small numbers a few records one way or the other may change the percentage value considerably, it has been noted as the records have accumulated that this value has tended to remain somewhere near 40 percent. For the present, therefore, until larger numbers have been obtained, it may be assumed that the crossover value of *I* and *A* is roughly 40 percent.

No special demonstration of the absence of crossing over in the female is necessary, since it is sufficiently demonstrated in the matings recorded in tables 1, 2 and 3. If crossing over were to occur in the female it would result in exceptions to the expected relations of sex and the sex-

[14] Only female offspring considered.

linked characters, but no such undoubted exceptions were found. Furthermore, crossing over in the female would make possible the occurrence of females homozygous for the sex-linked characters, and no such have ever appeared. The reported cases of exceptions in the dove and canary are doubtful, and if valid, are probably to be accounted for on another basis (BRIDGES 1916). GOODALE (1917) reports no crossing over in the female domestic fowl, so it seems legitimate to assume that it does not occur in birds. This means that if the sex chromosome of birds has a mate, as in Drosophila it does not bear any factors.

The best test of crossing over in the female is obtained when males recessive for both the sex-linked characters (*ia.ia*) are mated to females carrying them both (*IA*—). Under these conditions all the male offspring should carry both *I* and *A*, while the female progeny should be double recessive (*ia*). In our records there are three matings of this type. These are shown in table 4, and as far as they go they show results entirely in accord with the expectations on the assumption that crossing over does not take place in the female.

TABLE 4

Demonstrating absence of crossing over in the female.

Mating					Offspring		
Number	♂ parent		♀ parent		Non-crossover		Crossover
	Number	Formula	Number	Formula	♂ *IA*	♀ *ia*	
248	38 A	*ia.ia*	55 A	*IA*—	0	2	0
830	697 B	*ia.ia*	548 A	*IA*—	2	2	0
1470	1285 U	*ia.ia*	1333 A	*IA*—	7	8	0
Totals					9	12	0

Crossing over, 0 percent.

SUMMARY

1. Two sex-linked characters of the domestic pigeon have been studied, namely intensity of pigmentation (factor *I*), and an alteration in the appearance of the black pigment (factor *A*).

2. The *A* factor has a variable effect on the color of the bird, the differences depending, presumably, upon combinations of individual factors. There are apparently two main categories, ***dominant red*** and ***gray***. The dominant red presents an interesting contrast with the ***recessive red*** described in previous publications.

3. In the case of *I*, while the results were in accord with expectation as to the association of the character with sex, there was a considerable disturbance of the sex ratio, the males being much in excess of expectation. This seems to be due largely to excess of males in particular families, and may be the result of a recessive sex-linked lethal factor.

4. In the matings involving the *A* factor there was a deficiency rather than an excess of males. No explanation is apparent.

5. The two sex-linked factors *I* and *A* show a slight but appreciable mutual linkage. Crossing over in the male occurs in roughly 40 percent of the cases; there is no crossing over in the female.

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APPENDIX

Descriptions of dominant red and gray types

The following descriptions of individual birds selected to represent the more striking types of dominant reds and grays may be of value to the reader who desires to get a more definite notion of the colors referred to as resulting from the action of the *A* factor. Only brief and relatively incomplete descriptions are given at this time as it is planned to present much more detailed descriptions of these types after the genetic relations of the various conditions have been more thoroughly worked out. The color names in quotation marks are those given by RIDGWAY (1912).

1533B_2. *Dominant red, light rump.* Head, neck, breast, upper back and wings (except flights), dark reddish brown. Feathers taken from lesser wing coverts are slightly lighter than "Vandyke brown"—between that and "Rood's brown," but nearer the former.[15] On the head there is a slightly bluish cast, and the same is true on the lower breast and belly. Flights (except at tips) and primary coverts mealy (red and blue mixture). Tips of flights, rump and tail light steely blue ("gull gray"). In these parts there are prominent flecks of black.

1487B_2. *Dominant red, dark rump.* Distribution of red as in previous type; darker in color, apparently on account of numerous fine intermingled specks of black. Lesser wing coverts match very closely with RIDGWAY's "bister." Lower breast and belly very dark, almost black. Flights slaty (with reddish cast), not conspicuously lighter at tips. Rump and tail slaty, without the reddish. Rump a dark gray difficult to match, but somewhere near "dark mouse gray." Patches of black occur in upper tail coverts and tail, but not so many small flecks as in former case.

A80. *Red barred, clear.* Head, neck, upper breast and wing bars red. On the darkest part of the breast this is close to "burnt umber"; on the tertiaries, where it forms part of the wing bars, it is lighter, about "Vandyke brown." The proximal parts of the flights and their greater coverts are mealy. These mealy areas are continuous with the wing bars when the wing is open. Most other parts of the bird are a light bluish with a slightly reddish cast, the lesser wing coverts matching fairly well with "pallid mouse gray." The rump is lighter, almost white. Black flecking practically absent.

A7 shows some modification from this type. The distribution of red is less extensive, the head being a slaty blue (near "light neutral gray")

[15] The color of recessive reds (*ba* or *bA*) is lighter, commonly very close to "pecan brown" (COLE 1914, p. 320).

and practically free of red. The red of the breast is of about the same shade as in A80, but that of the wing bars is more brownish, the red bar on the tertiaries being close to "mass brown." Very little of the mealiness in the flights. The blue parts differ in lacking the warm tone of the other, owing to much less of the reddish cast mentioned. The color of the lesser wing coverts is nearly the same as is common on ordinary blues with black bars, namely "gull gray." Rump almost white. Black flecks and patches moderate in amount.

1456A. *Gray.* Fairly uniform gray, but darker on head, neck and breast, due in part to minute reddish specks in the feathers. The dark breast feathers are fairly near to "dark mouse gray" (not considering the inconspicuous brownish flecks). Lesser wing coverts "pale neutral gray." Incomplete reddish wing bars due to red-mealy patches in tertiaries and inner secondaries. Rump nearly uniform with back and tail. Numerous conspicuous black flecks and patches on nearly all parts.